

HAEMODYNAMIC STUDIES IN CIRRHOSIS

by
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DECLARATION OF ORIGINALITY

I declare that the work presented herein
and the composition of this thesis is my own.

Alison L Jones

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CONTENTS

Page

Abstract

List of figures

List of tables

List of appendices

Section I	Introduction	1
Section II	Portal and systemic haemodynamic response to acute and chronic administration of low and high dose isosorbide-5-mononitrate in patients with cirrhosis.	120
Section III	The effect of N-acetylcysteine on systemic and portal haemodynamics and oxygen delivery and consumption in patients with cirrhosis.	163
Section IV	The effect of N-acetylcysteine on forearm blood flow in patients with cirrhosis.	188
Section V	The pharmacokinetics of N-acetylcysteine in patients with cirrhosis.	206
Section VI	General discussion and conclusion	233
References		286
Appendices		347

ABSTRACT

Study of the haemodynamic response to drugs is important in evaluation of their ability to achieve reduction of oesophageal variceal bleeding and to optimise their haemodynamic action in patients with liver disease. My studies have concentrated on the evaluation of two drugs, N-acetylcysteine and isosorbide-5-mononitrate (a drug reported to have a problem of tolerance).

The portal and systemic haemodynamic response to low and high dose isosorbide-5-mononitrate (Is-5-Mn) was studied. 10 mg or 40 mg of oral Is-5-Mn was given acutely and chronically (bd for 4 weeks) allowing a 16 hour nitrate free interval to 25 patients with cirrhosis. Both doses of nitrate reduced the hepatic venous pressure gradient acutely and chronically, without evidence of tolerance, by a reduction in the wedged hepatic venous pressure. The effect on mean azygos blood flow was dependent on the initial azygos blood flow, not on the dose of nitrate used; if low then vasodilation was seen in response to Is-5-Mn and vice-versa. As the effect of Is-5-Mn on cardiac output, systemic resistance and its pharmacokinetics in the presence of chronic liver disease has been previously reported this was not studied further.

Intravenous N-acetylcysteine (NAC) was given to 11 patients with cirrhosis. There was no effect on mean heart rate or blood pressure despite a significant fall in systemic and pulmonary vascular resistance. Cardiac index increased but estimated liver blood flow and hepatic venous pressure gradients did not change

significantly. Administration of NAC increased oxygen delivery but not arteriovenous oxygen extraction ratio or mean tissue oxygen consumption.

Therefore NAC administration does not appear to confer haemodynamic benefit on patients with cirrhosis.

Studies using forearm blood plethysmography confirmed that intra-arterial administration of NAC causes local vasodilation in patients with cirrhosis and in normal controls.

Pharmacokinetic studies during intravenous NAC administration revealed that the area under the curve and therefore the half-life and clearance of the drug were significantly impaired in the presence of chronic liver disease. This contrasts with previous reports in acute liver damage due to paracetamol.

Mechanisms of nitrate and NAC action and tolerance are described.

LIST OF FIGURES		Page
Figure 1	The zones of the liver	4
Figure 2	The hyperdynamic circulation of cirrhosis	22
Figure 3	The collateral circulation	31
Figure 4	The layers of veins in the oesophagus	33
Figure 5	The concentration time curve for Hamiltons' dye dilution method for estimation of cardiac output	52
Figure 6	Schematic diagram of blood flow and pressure relationships to illustrate the effect of intra-abdominal pressure on portal pressure and variceal pressure	60
Figure 7	Theoretical effects of altering hepatic blood flow for a drug with a low intrinsic hepatic clearance and a drug with a high intrinsic hepatic clearance	112
Figure 8	Heart rate response to acute administration of 10 mg Is-5-Mn	132
Figure 9	Heart rate response to chronic administration of 10 mg Is-5-Mn	133
Figure 10	Heart rate response to acute administration of 40 mg Is-5-Mn	134
Figure 11	Heart rate response to chronic administration of 40 mg Is-5-Mn	135
Figure 12	Mean arterial blood pressure response to acute administration of 10 mg Is-5-Mn	136
Figure 13	Mean arterial blood pressure response to chronic administration of 10 mg Is-5-Mn	137
Figure 14	Mean arterial blood pressure response to acute administration of 40 mg Is-5-Mn	138
Figure 15	Mean arterial blood pressure response to chronic administration of 40 mg Is-5-Mn	139
Figure 16	The response of wedged hepatic venous pressure to 10 mg Is-5-Mn	140
Figure 17	The response of free hepatic venous pressure to 10 mg Is-5-Mn	142

Figure 18	The response of the hepatic venous pressure gradient to 10 mg Is-5-Mn	143
Figure 19	The response of wedged hepatic venous pressure to 40 mg Is-5-Mn	144
Figure 20	The response of free hepatic venous pressure to 40 mg Is-5-Mn	145
Figure 21	The response of the hepatic venous pressure gradient to 40 mg Is-5-Mn	146
Figure 22	Azygos vein blood flow response to acute administration of 10 mg Is-5-Mn	148
Figure 23	Azygos vein blood flow response to chronic administration of 10 mg Is-5-Mn	149
Figure 24	Azygos vein blood flow response to acute administration of 40 mg Is-5-Mn	150
Figure 25	Azygos vein blood flow response to chronic administration of 40 mg Is-5-Mn	151
Figure 26	The mean azygos blood flow response to Is-5-Mn - for three groups of initial azygos blood flows	152
Figure 27	The effect of 10 mg Is-5-Mn on estimated liver blood flow in 5 patients with cirrhosis	154
Figure 28	The effect of 40 mg Is-5-Mn on estimated liver blood flow in 7 patients with cirrhosis	155
Figure 29	The effect of N-acetylcysteine on mean arterial blood pressure in 11 patients with cirrhosis	169
Figure 30	The effect of N-acetylcysteine on mean heart rate in 11 patients with cirrhosis	170
Figure 31	The effect of N-acetylcysteine on mean systemic vascular resistance index in 11 patients with cirrhosis	171
Figure 32	The effect of N-acetylcysteine on mean pulmonary vascular resistance index in 11 patients with cirrhosis	172
Figure 33	The effect of N-acetylcysteine on mean estimated liver blood flow in 6 patients with cirrhosis	173

Figure 34	The effect of N-acetylcysteine on mean oxygen delivery in 11 patients with cirrhosis	175
Figure 35	The effect of N-acetylcysteine on mean cardiac index in 11 patients with cirrhosis	176
Figure 36	The effect of N-acetylcysteine on mean left ventricular stroke-work index in 11 patients with cirrhosis	177
Figure 37	The effect of N-acetylcysteine on the mean oxygen extraction ratio in 11 patients with cirrhosis	178
Figure 38	The effect of N-acetylcysteine on mean oxygen consumption in 11 patients with cirrhosis	179
Figure 39	Forearm blood flow in response to N-acetylcysteine - the control group	198
Figure 40	Forearm blood flow in response to N-acetylcysteine - the patients with cirrhosis	199
Figure 41	Forearm blood flow ratios (infused/control arm) in response to N-acetylcysteine - controls and patients with cirrhosis	200
Figure 42	Percentage change in forearm flow from baseline in response to N-acetylcysteine - controls and patients with cirrhosis	201
Figure 43	Semilogarithmic plot of serum N-acetylcysteine concentration against time for 6 healthy controls	217
Figure 44	Semilogarithmic plot of serum N-acetylcysteine concentration against time for 9 patients with cirrhosis	218
Figure 45	Semilogarithmic plot of serum N-acetylcysteine concentration against time for cirrhotic patients and normal controls	219
Figure 46	The two-compartment open system model	226
Figure 47	Nitric oxide as a vasodilator	248
Figure 48	N-acetylcysteine - possible mechanisms of action as a vasodilator	269

LIST OF TABLES**Page**

Table 1	The world-wide causes of cirrhosis	6
Table 2	Child's severity grading of cirrhosis - used for assessing prognosis of surgery	14
Table 3	Pugh's modification of Child's grading of cirrhosis	16
Table 4	The acute portal and systemic haemodynamic effect of nitroglycerin administration to patients with cirrhosis	84
Table 5a and 5b	The acute portal and systemic effect of isosorbide-dinitrate administration to patients with cirrhosis	87, 88
Table 6	The acute portal and systemic haemodynamic effect of isosorbide-mononitrate (Is-5-Mn) administration to patients with cirrhosis	91
Table 7	The chronic portal and systemic haemodynamic effect of oral isosorbide-dinitrate administration to patients with cirrhosis	93
Table 8	The chronic portal and systemic haemodynamic effect of oral isosorbide-mononitrate administration to patients with cirrhosis.	94
Table 9	Examples of commonly used drugs with high and low clearance by the liver	110
Table 10	A summary of the haemodynamic measurements made on each attendance at the catheter laboratory for the Is-5-Mn study	128
Table 11	Comparison of baseline haemodynamic variables between 10 mg and 40 mg Is-5-Mn study groups	131
Table 12	The effect of N-acetylcysteine on mean wedged and free hepatic venous pressure and hepatic venous pressure gradient in 6 patients with cirrhosis	174
Table 13	The effect of N-acetylcysteine on mean blood gas values in 11 patients with cirrhosis	180
Table 14	The haemodynamic response to dextrose infusion without N-acetylcysteine (control) in 5 patients with cirrhosis	182

Table 15	Subject characteristics for the forearm blood flow study	190
Table 16	Baseline variables - patients with cirrhosis and healthy controls	210
Table 17	Pharmacokinetic variables - healthy controls	220
Table 18	Pharmacokinetic variables - patients with cirrhosis	221
Table 19	A comparison of the pharmacokinetic variables between patients with cirrhosis and normal controls	222
Table 20	Drug combinations with probable synergistic effects in reducing portal pressure	255

LIST OF APPENDICES**Page**

Appendix Ia	Patient details 10 mg Is-5-Mn study group	348
Appendix Ib	Patient details 40 mg Is-5-Mn study group	349
Appendix II	Comparison of baseline patient characteristics between the 10 mg and 40 mg Is-5-Mn study groups	350
Appendix III	Sample of free and wedged hepatic venous pressure traces	351
Appendix IV	Indocyanine green concentrations in serum as measured by optical density at 805nm	352
Appendix V	Anatomical position of the azygos vein	353
Appendix VIa & b	A sample azygos vein blood flow recording	354, 355
Key to Appendices VII to X		356
Appendix VII	Change of haemodynamic parameters from baseline value in response to 10 mg Is-5-Mn	358
Appendix VIII	Matrix of Spearmans' Rank Correlations between changes in haemodynamic variables and plasma bilirubin, albumin, alanine aminotransferase and prothrombin ratio in the 10 mg Is-5-Mn group	359
Appendix IX	Change of haemodynamic parameters from baseline value in response to 40 mg Is-5-Mn	360
Appendix X	Matrix of Spearmans' Rank Correlations between changes in haemodynamic variables and plasma bilirubin, albumin, alanine aminotransferase and prothrombin ratio in the 40 mg Is-5-Mn group	361
Appendix XI	N-acetylcysteine study patient details	362
Appendix XII	Summary of formulae used in haemodynamics calculations	363
Key to Appendices XIII and XIV		365
Appendix XIII	Change of haemodynamic parameters from baseline value in response to N-acetylcysteine	366

Appendix XIV	Matrix of Spearmans' Rank Correlations between changes in haemodynamic variables and plasma bilirubin, albumin, alanine aminotransferase and prothrombin ratio in response to N-acetylcysteine	367
Appendix XV	Patient details (cirrhotic group) in the forearm blood flow study	368
Appendix XVI	Subject details (normal controls) in the forearm blood flow study	369
Appendix XVII	Forearm blood flow response to N-acetylcysteine - control group	370
Appendix XVIII	Forearm blood flow response to N-acetylcysteine - patients with cirrhosis	371
Appendix XIX	Forearm blood flow ratios (infused/control arm) in response to N-acetylcysteine in cirrhotic patients compared with a control group	372
Appendix XX	Percentage change in forearm flow from baseline in response to N-acetylcysteine in cirrhotic patients compared with a control group	373
Appendix XXI	Patient details - pharmacokinetic study	374
Appendix XXII	Sample HPLC trace showing glycylglycine and N-acetylcysteine peaks eluted	375
Appendix XXIII	Estimates of serum N-acetylcysteine at time points for each subject	376
Appendix XIV	Pharmacokinetic analysis for each subjects' decay curve of serum N-acetylcysteine concentration against time	378
Key to Appendix XXV		393
Appendix XXV	Correlation of Child's-Pugh variables and pharmacokinetic parameters (Spearman Rank Correlation)	394
Appendix XXVI	Experimental data, fitting a calculated curve and determining the equation for a pharmacokinetic study	395
Appendix XXVII	Publications to date	396

Section I

Introduction

"If oesophago-gastric varices did not form and bleed portal hypertension would be of virtually no clinical significance"

(Dame Sheila Sherlock 1993)

Unfortunately, they do form and bleed and this presents one of the major clinical problems in hepatology. Cirrhosis, the commonest cause of portal hypertension, is a leading cause of death in the Western world being the ninth most common cause of death in the United States (Grant et al, 1991).

CIRRHOSIS

DEFINITION

Cirrhosis is defined as widespread fibrosis and nodule formation within the liver. It follows hepatocellular necrosis due to a variety of insults and reflects the fact that the liver's response to necrosis is limited. The distribution of the fibrous septa tends to vary according to the causative agent; in haemochromatosis there is portal zone or Zone 1 fibrosis, whereas in alcoholic cirrhosis, the fibrosis is predominantly in Zone 3 (Figure 1).

The fibrosis disrupts hepatic architecture, impeding exchange of oxygen and nutrients through the basement membranes between liver cells and the blood, and causing portal hypertension (Shibayama et al, 1989). Fibrous tissue is not just a structural support for damaged hepatocytes but may modulate hepatocyte patterns of function (Biagini and Ballardini, 1989).

Cirrhosis is usually believed to be irreversible, but fibrosis has been shown to regress in treated haemochromatosis and Wilson's disease and the concept of reversibility is therefore an exciting therapeutic goal.

Three anatomical categories of cirrhosis are recognised: micronodular, macronodular and mixed, although this distinction is rather arbitrary. Thick septae divide small regenerating nodules in micronodular cirrhosis and macronodular cirrhosis is characterised by septa and nodules of various sizes.

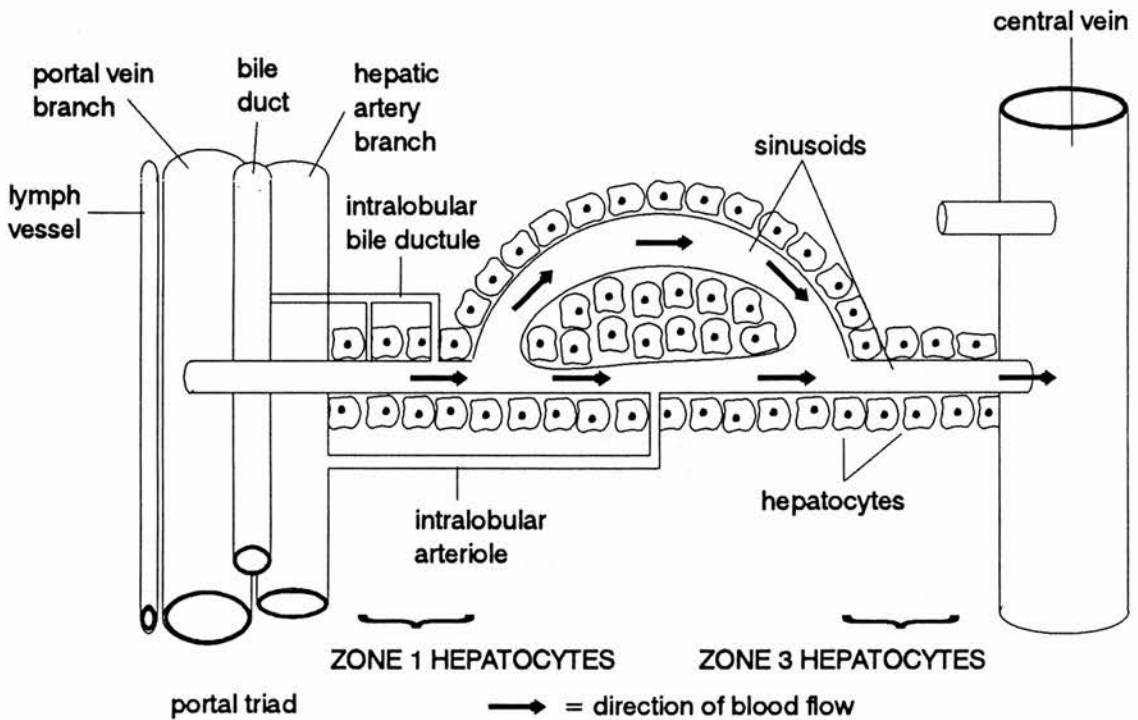


Figure 1

The zones of the liver

Zone 1 occurs around the portal triad which contains the portal vein, hepatic artery and bile duct.

Zone 3 is near the circulatory periphery: this zone suffers most from injury whether viral, toxic or anoxic.

(After Timbrell, 1991)

AETIOLOGY OF CIRRHOSIS

There are many causes of liver cirrhosis, some of which are shown in Table 1.

The commonest cause world-wide is hepatitis B infection but in the United Kingdom is excessive alcohol consumption.

Aetiological distinction is important both for prognosis and treatment.

Hepatitis B and C are not discussed here as patients known to have viral hepatitis or cirrhosis were excluded from this study because of the potential infection risk to staff.

Alcohol

Matthew Baillie in 1793 is credited with being the first person to report an association between alcohol consumption and cirrhosis.

In 1956, the French mathematician Ledermann hypothesised that per capita alcohol consumption was a good indicator of the proportion of heavy drinkers in a given population, and that alcohol-related problems including cirrhosis increased with per capita consumption (Ledermann, 1956).

Numerous epidemiological studies have since confirmed a strong association between per capita alcohol consumption and the morbidity and mortality from cirrhosis. Cirrhosis mortality abruptly decreased in Europe after the First and Second World Wars, when rationing of alcohol was in force, and during the prohibition era (1919-32) in the United States.

Table 1

Aetiology of cirrhosis (world-wide)

Alcohol

Cholestasis (intra- and extra-hepatic)

Cryptogenic (of unknown aetiology = 10-15% cirrhosis in United Kingdom)

Hepatic venous outflow block:

- Budd Chiari syndrome
- Constrictive pericarditis
- Heart failure
- Veno-occlusive disease

Immunological (chronic active hepatitis)

Metabolic:

- Alpha-1-antitrypsin deficiency
- Copper overload (Wilson's disease)
- Diabetes mellitus
- Galactosaemia
- Type IV glycogen storage disease
- Iron overload (haemochromatosis)
- Tyrosinaemia

Schistosomiasis

Toxins and drugs e.g. methotrexate, amiodarone

Viral hepatitis (B, C and D)

Unfortunately world-wide alcohol consumption is increasing and countries such as Japan are now discovering that alcoholic cirrhosis, once a rarity, has become a significant cause of morbidity and mortality (Ohnishi and Okuda, 1986).

Cirrhosis is the most severe form of alcoholic liver injury and characteristically is of the micronodular type (Leevy, 1968; Saunders et al, 1985; Sherlock and Dooley, 1993). The first stage of hepatic damage by alcohol appears to be formation of a fatty liver. Thereafter alcoholic hepatitis and perivenular fibrosis are thought to develop into cirrhosis. Whether an individual has to pass through all these stages in the development of cirrhosis is controversial.

Not everyone who abuses alcohol develops liver damage; it is estimated that at least 80% of heavy drinkers show some features of fatty liver, 10-35% develop alcoholic hepatitis and approximately 10% will develop cirrhosis (Grant et al, 1988).

A retrospective study in men showed that 50% of men with an average intake of alcohol of greater than 160 g (approximately 16 units) per day for 20 years developed cirrhosis (Lelbach, 1975). Fifty-two per cent of a predominantly male group of patients, drinking in excess of 80 g of alcohol per day, developed cirrhosis over a 20-year period (Saunders, 1985). Later studies have suggested risk levels as low as 40 g alcohol per day for men (Pequinot et al 1974; Pequinot et al, 1978; Batey et al, 1992).

The duration of alcohol abuse appears important. Neither cirrhosis nor alcoholic hepatitis were seen in patients who consumed an average of 160 g of

ethanol per day for less than five years, whereas 50% of patients consuming these levels of alcohol for an average of 21 years developed cirrhosis (Leibach et al, 1975).

Liver injury appears not to be related to the type of beverage; only to its alcohol content. Regular drinking appears more dangerous than intermittent drinking, since presumably in the latter the liver has greater chance to recover from the toxic effects of ethanol. In a UK study of problem drinkers the risk of cirrhosis was 12 times greater in those drinking 6-7 days per week than those who drank on 1-3 days per week, after controlling for cumulative alcohol consumption. Over 90% of cirrhotic patients had a pattern of unremitting daily drinking, whereas those who showed minor abnormalities mostly had an intermittent pattern of consumption (Saunders et al, 1985).

Sex-related differences in alcoholic liver disease were first reported by Spain (1945) and there is now a large body of evidence indicating that women are more susceptible than men to alcohol-related liver disease. They tend to develop cirrhosis at a younger age, at lower levels of alcohol consumption (exceeding 20 g per day, compared with over 40 g per day for men) and after a shorter periods of alcohol abuse (by approximately 6 years) (Wilkinson et al, 1969; Morgan and Sherlock, 1977; Saunders et al, 1981; Bhattacharayya and Rake, 1983; Norton et al, 1987).

Alcoholism is increasing among women as the social stigma surrounding drinking declines and the access to alcohol becomes easier. However, they are

less likely to be suspected of alcohol abuse; they present at a more advanced stage of disease, and are more likely to relapse after treatment (Morgan and Sherlock, 1977). They are also more likely to progress from alcoholic hepatitis to cirrhosis even if they stop drinking (Pares et al, 1986). The increased vulnerability of women to alcohol-induced liver injury has been attributed to significantly higher blood alcohol concentrations and area under the concentration-time curve that are attained compared with men after consumption of the same amount of alcohol. Women often weigh less than men and have therefore a lower volume of distribution for alcohol.

Different rates of alcohol elimination between individuals (and consequently risk of toxic effects of alcohol) may be related to genetic polymorphism of two enzyme systems, MEOS (microsomal ethanol oxidising system) and ADH (alcohol dehydrogenase), which metabolise alcohol. Genetic polymorphism for ADH has been found at two gene loci and differences have been shown to alter rates of ethanol oxidation and acetaldehyde generation (Bosron and Li, 1986).

Acetaldehyde, the principle metabolite of alcohol, is metabolically extremely reactive and hepatotoxic (Sherlock, 1993). Alcoholics have higher than normal blood acetaldehyde levels after alcohol (Peters and Ward, 1988). This might be secondary to enzyme induction or a primary genetic abnormality.

In addition an increased prevalence of histocompatibility antigens HLA-B8, HLA-B13 and HLA-B40 has been reported in patients with alcoholic cirrhosis compared with non-alcoholic controls. The presence of HLA-B8 has been

linked to more rapid progression of liver injury and development of cirrhosis in both men and women (Saunders et al, 1982) but it is unclear whether this reflects an association with excessive alcohol consumption or an increased susceptibility to alcoholic liver disease.

It has been postulated that concurrent infection with viral hepatitis B and/or hepatitis C increases susceptibility to alcohol induced liver damage and worsens the prognosis.

Patients with alcoholic cirrhosis have a high prevalence of serum antibodies to hepatitis B virus (Mills et al, 1981) and an association between hepatitis B surface antigen positivity and a tendency to severe alcoholic liver disease has led to the suggestion that alcohol and hepatitis B infection may act synergistically to produce liver damage (Chung et al, 1989; Villa et al, 1982). However, another study showed no such correlation (Mendenhall et al, 1991), so the association is controversial.

In contrast, a significant correlation has been demonstrated between antibodies to hepatitis C virus and alcoholic liver disease. Hepatitis C antibodies are more prevalent in patients with severe alcoholic liver disease (Pares et al, 1990). These findings are particularly relevant to Japan where there is a high prevalence of both hepatitis B and hepatitis C, and where the incidence of alcoholic liver disease is rapidly increasing in association with increasing per capita consumption of alcohol (Ohnishi and Okuda, 1986).

Much also depends on nutrition; alcohol given to rats does not produce liver damage unless accompanied by a diet deficient in essential nutrients particularly choline. In rhesus monkeys the liver damage induced by alcohol could be prevented by increasing dietary protein and choline (Rogers et al, 1979). Certainly patients with decompensated liver disease, given a third of their calories as alcohol together with a nutritious diet, improve steadily (Reynolds et al, 1965), whereas liver function does not improve with alcohol abstinence if dietary protein remains low (Phillips et al, 1952). It seems likely that both alcohol and nutrition play a part in alcohol hepatotoxicity, alcohol being the more important.

Primary biliary cirrhosis

Primary biliary cirrhosis was first described in 1851 by Addison and Gull and is a disease of unknown cause in which intra-hepatic bile ducts are progressively destroyed. Ahrens et al gave the name primary biliary cirrhosis (PBC) to the condition in 1950. It is associated with a marked immunological disturbance which has been related to the degree of bile duct destruction (Fox et al, 1969; Gershwin and Mackay, 1991), the final event being an attack by cytotoxic T-cells. The trigger for the immune cascade may be viral, bacterial or defective immune regulation.

Circulating antibodies against mitochondria are found in virtually 100% of patients with PBC (Walker et al, 1965; Munoz et al, 1981). They are not organ specific but are directed at the inner mitochondrial membrane at the pyruvate dehydrogenase complex. Currently, the presence of anti-mitochondrial

antibodies is detected by indirect immunofluorescence on rat kidney substrate but it is expected that a more specific and sensitive anti-M2 subtype of antimitochondrial antibody test will be available soon for diagnostic purposes.

The prevalence of circulating antimitochondrial antibodies is increased in relatives of patients (Feizi et al, 1972; Galbraith et al, 1974; Klein and Berg, 1990). PBC has been reported in sisters, twins, mothers and daughters and other family clusters (Chohan, 1973; Tong et al, 1976).

It has been postulated that cross-reactivity of the antigens between bile ducts and micro-organisms may account for the pathogenesis of PBC (Burroughs et al, 1984).

90% of patients are female, usually between 40 and 59 years of age. The disease starts insidiously with pruritis without jaundice, the latter appearing within six months to two years. In 25% of patients both start together. Many patients are now diagnosed asymptotically by automated liver function tests and they usually survive at least 10 years and for some life-expectancy is no different from the general population (Beswick et al, 1985). In those with symptomatic disease and jaundice the survival is about seven years (Sherlock, 1959) and serum bilirubin values are good indicators of prognosis (Shapiro et al, 1979; Epstein et al, 1985).

Treatment for PBC includes general measures such as control of itch.

Ursodeoxycholic acid improves liver function but does not increase survival (Heuman et al, 1991). PBC is sometimes the commonest and often the second

most frequent indication for hepatic transplantation in adults. There does however appear to be a risk of recurrence of the disease in the transplanted liver.

A host of associations with PBC are reported including rheumatoid arthritis, dermatomyositis, mixed connective tissue disease, CREST syndrome and systemic lupus erythematosus.

Secondary biliary cirrhosis

One patient in our study had secondary biliary cirrhosis as a result of bile duct surgery. He had no history of alcohol consumption.

PROGNOSIS OF CIRRHOSIS

Liver cirrhosis results in two major complications; hepato-cellular failure and portal hypertension. Treatment and prognosis depend on both these factors, the latter being the more important. The introduction of liver transplantation has emphasised the need for accurate prognosis so that surgery, if indicated, may be performed at the right time. Of the systems designed to assess prognosis, two have been widely used - Child's grading and Pughs modification of Child's grading. In 1964 Child and Turcotte introduced a system of grading to assess the risk of surgery in patients with liver disease: It was based on five variables - serum concentrations of bilirubin and albumin, the absence or extent of ascites and of neurological disorder, and the nutritional status (Table 2). The main problem with this grading system is that the findings in an individual patient may be a mixture of those in columns A, B and C.

Table 2

Child’s severity grading of cirrhosis: used for assessing prognosis of surgery

Clinical and biochemical measurements	Child’s grade		
	A	B	C
Serum bilirubin (mg/100ml)	< 2.3	2.3 - 2.9	> 2.9
Serum albumin (g/L)	> 35	30 - 35	< 30
Ascites	absent	easily controlled	poorly controlled
Encephalopathy	none	minimal	coma
Nutrition	good	moderate	poor, wasting

(Child & Turcotte, 1964)

Pugh and his colleagues (1973) therefore modified Child's grading by adding prolongation of the prothrombin time to the list of variables and giving a score to each observation and summing of the scores in order to obtain an overall result (Table 3). This modified score remains a very useful method of assessing prognosis in cirrhosis (Infante-Rivard et al, 1987). The one-year survival in good-risk (Child A and B) patients is about 70% and in poor-risk (Child C) patients about 30%.

Cox's regression model using proportional hazards has been applied to primary biliary cirrhosis and a prognostic index formulated (Christensen et al, 1986). Poor prognosis is associated with a low prothrombin index, marked ascites, gastrointestinal bleeding, advanced age, high daily intake of alcohol, high serum bilirubin and alkaline phosphatase, low albumin values and poor nutrition.

Study of a very large number of Italian patients with cirrhosis showed that decompensation (i.e. onset of jaundice and ascites) developed at a rate of 10% per year. Only 20% of decompensated patients survived six years. Significant mediators of death were advanced age, male sex, encephalopathy, variceal haemorrhage, varices, prothrombin time, hepatitis B surface antigen positivity and hepatocellular carcinoma (D'Amico, 1986).

Alcoholics, if they continue drinking, tend to have the worst prognosis as hepatocellular disease is greater. If they abstain however, their prognosis is better than that of patients with "cryptogenic" cirrhosis, ie cirrhosis of unknown aetiology. Prognosis is also better if hepatic decompensation has followed a

Table 3

Pugh’s modification of Child’s grading of the severity of cirrhosis

Clinical and biochemical measurements	Score 1 point	Score 2 points	Score 3 points
Serum bilirubin (umol/L) (in primary biliary cirrhosis)	17 - 34 (17 - 68)	34 - 51 (68 - 110)	> 51 > 110
Prolongation of prothrombin time (seconds)	1 - 4	4 - 6	> 6
Serum albumin (g/L)	> 35	28 - 35	< 28
Ascites	none	slight	moderate or more
Encephalopathy grade	none	1 and 2	3 and 4

Child’s-Pugh Grade A = 5 or 6 points
Child’s-Pugh Grade B = 7 to 9 points
Child’s Pugh Grade C = 10 to 15 points

Minimum score = 5 points; Maximum = 15 points

(Pugh et al, 1973)

correctable factor, such as gastrointestinal haemorrhage, rather than being spontaneous. Patients who fail to improve within one month of starting hospital treatment, have a poor outlook and persistent hypotension (BP systolic < 100 mmHg) is also an adverse prognostic factor.

THE PORTAL VENOUS SYSTEM

The portal system includes all veins that carry blood from the abdominal part of the alimentary tract, the spleen, pancreas and gallbladder. The portal vein is formed by the union of the superior mesenteric and splenic veins just immediately anterior to the head of the pancreas at about the level of the second lumbar vertebra. The portal vein enters the liver at the porta hepatis before dividing into two main branches. It has no valves in its channels. Within the liver it has a segmental distribution accompanying the hepatic artery.

The liver has a vast anastomotic vascular network, the large cross section of the vessels producing a low resistance system. The hepatic vascular bed is expandable, for example when a large increase in portal blood flow occurs postprandially.

Portal blood flow in man is about 1.0-1.2 L/minute at rest and contributes 72% of the total oxygen supply of the liver. Hepatic arterial pressure is approximately 90 mmHg. The hepatic artery supplies the remainder with a flow of approximately 350 mL/minute. Portal pressure is normally 6-8 mmHg and hepatic vein pressure 2-4mmHg in man (Richardson and Withrington, 1981; Hobsley, 1982; Sherlock and Dooley, 1993).

Occlusion of the hepatic artery results in a fall in hepatic portal venous resistance and increase in portal flow while conversely, reduction in the portal inflow increases hepatic arterial blood flow termed reciprocity (Richardson and Withrington, 1981). This intrinsic regulation of liver blood flow is postulated to be due to either metabolic or myogenic mechanisms.

The metabolic hypothesis regards tissue oxygen supply as the regulated factor. When tissue oxygen supply fails to meet demand, vasodilator metabolites are released causing resistance causing vasodilation. Postulated mediators include hydrogen ions (Cohn and Kountz, 1963; Gelman and Ernst, 1977), increased plasma osmolality (Lautt et al 1977; Richardson and Withrington, 1980), adenosine and all the phosphates of adenosine (ATP,ADP,AMP) (McCuskey, 1966; Su, 1978).

The myogenic hypothesis considers local intravascular pressure to be the important regulated variable and it proposes that an increase in the transmural pressure gradient across the arterioles stretches the vascular smooth muscle cells which react by contracting and bring about an increase in vascular resistance, thereby maintaining a constant blood flow in the face of increased arterial pressure (Richardson and Withrington, 1981).

Whether autoregulation, in the sense of pressure-dependent control by blood flow, occurs in the liver is controversial. Some studies have shown small degrees of autoregulation in the hepatic arterial bed (Torrance, 1961; Hanson and Johnson, 1966). Autoregulation appears to be more pronounced in fed

than in fasted animals (Norris et al, 1979; Granger and Norris, 1980). There is no such autoregulation in the portal vascular bed (Richardson and Withrington, 1981).

The control of the portal flow is not inherent to the liver but is due to altered resistance of the vessels of the splanchnic organs that supply the portal system. This concept that the portal inflow is controlled by the splanchnic arterioles and not by the hepatic vessels is crucial to understanding the pathophysiology of portal hypertension.

PORTAL HYPERTENSION

As early as 1748 it was noted that the portal vein, spleen and haemorrhoids were related in an ill-defined syndrome (Stahl, 1748). Bleeding from oesophageal varices was first reported in 1840 (Power) but its association with cirrhosis was not appreciated until 1858 (Fauvel). McIndoe first used the term "portal hypertension" in 1928 and observed progressive dissociation of the portal venous and hepatic arterial circulations resulting in diversion of portal blood into collateral channels at an earlier stage than arterial blood (McIndoe, 1928). The hepatic cells thus came to be nourished almost entirely by the hepatic artery.

The first measurements of portal pressure were made by Caughey in 1936, who recorded an increase in splenic venous pressure in patients with Banti's syndrome and thus established portal hypertension as a clinical entity (Rousselot, 1936).

HAEMODYNAMIC CHANGES IN CIRRHOSIS

Forwards and backwards (resistance) flow theories of portal hypertension

Portal hypertension is thought to result from an increased resistance to portal venous blood flow through the liver ("backwards"/resistance mechanism) and/or raised portal venous blood flow through the liver ("forward flow mechanism").

Resistance factor

Although increased splanchnic flow is undoubtedly a factor in the development of portal hypertension, increased intrahepatic resistance to blood flow is at least as important and the site of this is the basis for the anatomical classification of portal hypertension into presinusoidal, sinusoidal and post-sinusoidal types (Groszmann et al, 1979; Groszmann, 1984). Progressive hepatic disease may produce more complex flow patterns in which the presinusoidal, sinusoidal and postsinusoidal patterns merge to produce a mixed type of lesion.

The precise cellular or subcellular location of the resistance in intrahepatic portal hypertension is not well defined. In 1963, Schaffner and Popper used electron microscopy to study biopsies from 63 patients and showed that the space of Disse had increased collagen, sinusoidal lining cell proliferation and a structure similar to a basement membrane. These findings supported the theory of a sinusoidal location of resistance to intrahepatic blood flow. However Viddins et al (1985) showed that in liver biopsies from patients with alcohol induced cirrhosis there was a marked reduction in sinusoidal area when

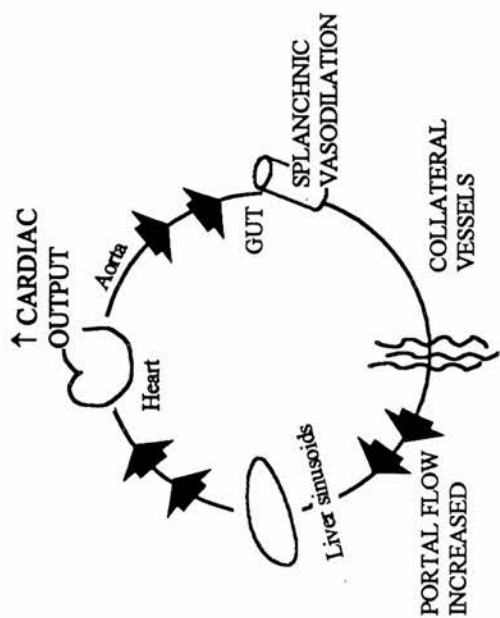
compared to normal biopsies. Furthermore, when the sinusoidal area was diminished by greater than 80%, an inverse relationship with hepatocyte size was noted and they concluded that hepatocyte expansion (i.e. ballooning) with compression of the sinusoids was important in the aetiology of increased portal pressure in severe alcoholic liver disease. Other studies have shown the presence of myofibroblast-like cells surrounding the sinusoids and hepatic venules.

Using isolated liver perfusion studies Bhatal and Grossman (1985) have demonstrated that a reversible component of hepatic resistance in cirrhotic livers and that vasodilators could reduce hepatic resistance i.e that it may be amenable to pharmacological treatment.

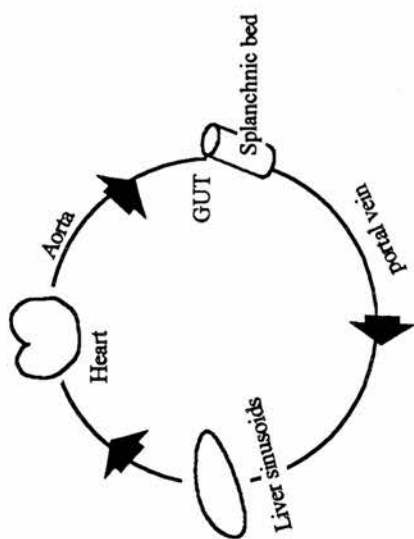
Forward flow factor

Portal hypertension is associated with a hyperdynamic splanchnic circulation characterised by low splanchnic arteriolar resistance and increased portal venous inflow in experimental models of portal hypertension (Vorobioff et al, 1984) and in humans (Kotelanski et al, 1972) (Figure 2).

The increased blood flow is not limited to the splanchnic territory; high cardiac output and low systemic vascular resistance characterises the circulation in cirrhosis. These findings have been confirmed in the rat model and in cirrhotic patients assessed by Doppler ultrasound and the systemic change occurs earlier than the splanchnic one (Colombato et al, 1992), possibly because the increased outflow resistance induced by portal hypertension delays



Forward flow theory
of portal hypertension



Normal portal circulation

Figure 2

The hyperdynamic circulation of cirrhosis

(by a myogenic reflex) the effect of circulating humoral vasodilators on the splanchnic bed.

Evidence for both mechanisms in portal hypertension

Sikuler and Groszmann (1985) studied the evolution of portal hypertension in rats with partial portal vein constriction, a model of portal hypertension that mimics the haemodynamic changes seen in humans. Rapid changes occurred including portal-systemic shunting, a decrease in splanchnic arteriolar resistance and an increase in portal blood inflow. The latter contributes to portal pressure and the maintenance of portal hypertension. Therefore, although the initial pathological insult causes increased resistance, portal hypertension is partially maintained by increased blood flow.

The mechanisms underlying the hyperdynamic circulation are under intense investigation. Multiple theories have been proposed including arteriovenous shunting and alterations in the sympathetic nervous system. Several vasoactive substances have also been suggested as being responsible, including glucagon (Kock et al, 1970; Benoit et al, 1984), bile acids (Ohkubo et al, 1984; Benoit et al, 1989), prostaglandins or prostacyclin (Zipser et al, 1979; Bruix et al, 1985; Blanchart et al, 1985; Sitzman et al, 1989), 5-hydroxytryptamine (Cummings et al, 1986), gamma-aminobutyric acid (Minuk and MacConnell, 1988), and nitric oxide (Vallance and Moncada, 1991). Glucagon and bile acids have been found in high concentrations in blood from various models of portal hypertension and in patients with chronic liver disease and are potent splanchnic vasodilators in

animals but neither consistently dilates other vascular beds (Korthuis et al, 1985; Genecin et al, 1990).

Prostaglandins E1 and E2 and prostacyclin have vasodilator effects. In humans with cirrhosis, urinary prostaglandin E excretion is enhanced. In portal vein ligated rats, portal vein prostaglandin activity is increased; however, no effect on systemic or splanchnic haemodynamics was derived from the suppression of prostaglandins by indomethacin (Blanchart et al, 1985).

Evidence for a hormonal agent is provided by several rat studies.

Cross-perfusion of intestinal vessels between normal rats and those with induced portal hypertension revealed that the vasodilation of the intestinal arteries was transferable (Benoit et al, 1984). Likewise, a study of cross-perfusion between the hindquarters of non-cirrhotic rats (without livers) and those with induced portal hypertension revealed increased blood flow and decreased resistance in the non-cirrhotic partners (Korthuis et al, 1985).

When the liver was included in both circulations in such parabiosis experiments however no evidence of a transferable humoral factor was found (Sikuler and Groszmann, 1985), ie this may be due to dilution and subsequent ineffective plasma concentrations of vasoactive substances or metabolism of the substance by the normal liver suggesting that the substance(s) that mediate the haemodynamic circulation depend on the existence of substantial functional or anatomic portal-systemic shunting.

More recently, the results of several studies suggest that nitric oxide, a powerful endogenous vasodilator secreted by endothelial cells, may be involved in inducing the vasodilatation seen in chronic liver disease (Pizcueta et al, 1992; Sieber and Groszmann, 1992).

The role of nitric oxide in portal hypertension and the development of the hyperdynamic circulation of cirrhosis

Nitric oxide (NO), a vasodilator synthesised from L-arginine by vascular endothelial cells (Palmer et al, 1988), accounts for the biological activity of endothelium-derived relaxing factor (Palmer et al, 1987) which is said to play a crucial role in blood pressure regulation (Rees et al, 1989). Released by vascular endothelium, NO diffuses into vascular smooth muscle and results in relaxation via the activation of cyclic GMP (Moncada and Higgs, 1993). Blood flow and O₂ tension are known to be potent stimuli for NO secretion (Miller et al, 1986).

Vallance and Moncada (1991) first suggested that NO may have a role in the hyperdynamic circulation of cirrhosis. Blockade of NO increases MABP and systemic vascular resistance and decreases cardiac output in normal rats (Lee et al, 1992; Pizcueta et al, 1992; Claria et al, 1992; Haylor et al, 1991). Inhibition of NO synthesis by N-omega-nitro-L-arginine (NNA) has been shown to restore the vascular hyporesponsiveness to methoxamine in portal hypertensive rats (Lee et al, 1992; Sieber and Groszmann, 1992; Sieber et al, 1993). In addition, short-term administration of either N-omega-nitro-L-arginine or NG-monomethyl-L-arginine (L-NMMA), another NO synthase inhibitor

attenuates the hyperdynamic splanchnic and systemic circulation observed in portal hypertensive rats (Lee et al, 1993 a and b; Pizcueta et al, 1992 respectively). Wu et al (1993) measured aortic, central and portal venous pressures directly in portal hypertensive rabbits and found that L-NMMA produced superior mesenteric artery vasoconstriction; indomethacin induced further constriction implying that NO and prostaglandins probably act as vasodilators by independent mechanisms. In vitro hyporesponsiveness to angiotensin II in aortic rings of cirrhotic rats with ascites was reversed on endothelium denudation or NO synthesis inhibition with N-omega-nitro-L-arginine (Castro et al, 1993).

Although previous studies in experimental portal hypertension have suggested that NO plays a role in the development of portal-systemic shunting, our work with patients with cirrhosis showed that administration of L-NMMA to patients with cirrhosis had no effect on azygos vein blood flow but did modify the systemic circulation by reduction of heart rate and elevation of blood pressure (Forrest et al, 1994). In addition, Calver et al (1994) demonstrated that nitric oxide inhibition with L-NMMA did not reverse the vasodilation seen in a forearm blood flow model of cirrhosis. The extent to which NO is responsible for the haemodynamic changes that accompany cirrhosis thus remains controversial.

The unstable nature of nitric oxide precludes its easy measurement; however it is rapidly converted to serum nitrite and nitrate which can be measured. A recent study has found that increased serum levels of nitrite and nitrate are

found in patients with cirrhosis, particularly those with ascites and may be a consequence of enhanced NO production in these patients (Guarner et al, 1993). However the finding could also be explained by dietary intake of proteins or diminished renal excretion of nitrite/nitrate.

Role of endothelins in cirrhosis

Endothelins are small peptides, mainly synthesised and released by endothelial cells, that act on adjacent smooth muscle cells in a paracrine fashion. They appear to be involved in the control of cardiovascular and renal function and act as neuromodulators.

Endothelin-1 was first isolated in 1988 from the supernatants of endothelial cells in culture (Yanagisawa et al, 1988). Two homologous forms have since been identified; endothelin-2 and 3 (Inoue et al, 1989). Many factors are known to influence the production of endothelins by cells including endotoxin, adrenaline, angiotensin II and arginine-vasopressin (Yanagisawa M et al, 1988; Emori et al, 1989; Sugiura et al, 1989). It is the most potent vasoconstrictor known to date. When it was infused in vivo to animals or man an intense sustained vasoconstriction followed an initial transient decrease in blood pressure (resulting from stimulation of the endothelial vasodilators nitric oxide and prostacyclin) (Miller et al, 1989; Vierhapper et al, 1990; deNucci et al, 1988). Endothelin-induced vasoconstriction appears to be due to a direct effect on vascular smooth muscle cells and is reversed by nitric oxide; so there appears to be a fine interplay between the nitric oxide and endothelins in the control of vascular tone.

Current information concerning the role of endothelins in cirrhosis is conflicting. Six studies investigating the plasma concentration of endothelin in cirrhosis have been published to date (Schrader et al, 1990; Moore et al, 1991; Lerman et al, 1991; Veglio et al, 1992; Uchinara et al, 1993 and Asbert et al, 1993). The studies show marked differences; only some show elevated plasma levels of endothelins in patients with ascites. The discrepancies between these studies are difficult to explain but neither the sodium intake or diuretics are mentioned in many of them. In the study of Asbert et al (1993) control subjects and cirrhotic patients were studied in conditions of identical sodium intake and elevated plasma endothelin levels were found in cirrhotic patients with ascites than controls.

This may be due to increased endothelial synthesis of endothelin in cirrhosis as a compensatory mechanism to antagonize the effects of vasodilator factors of endothelial origin eg nitric oxide or prostaglandins. Future studies using specific antagonists of endothelin will it is hoped lead to a better understanding of the pathophysiological role of endothelin on systemic and renal haemodynamic and neurohumoural changes in cirrhosis.

The balance of forces in the hyperdynamic circulation of cirrhosis

Generalised vasodilation, probably mediated by a combination of the factors already described, characterises the haemodynamics of portal hypertension.

What is not clear, however, is the event that leads from this vasodilation to the state of high cardiac output and increased regional blood flow, since

vasodilators given to controls produce only a *transient* increase in cardiac output and regional blood flow, which usually does not persist.

It is suggested that for a hyperdynamic state to develop and persist requires not only reduced cardiac afterload, but also an increased venous return. Genecin et al (1990) and Albillos et al (1992) reported an expanded plasma volume after initial vasodilation in rats with portal hypertension. They also found amelioration of the hyperdynamic circulation after sodium restriction and suggested that this "refill" of the circulation may be intrinsic to the development of the hyperdynamic state and that blood volume expansion may be the link between vasodilation and the hyperdynamic circulation.

In preascitic cirrhosis, the renin-angiotensin and sympathoadrenergic systems do not differ from healthy controls either in the upright or supine position, suggesting that the effective arterial blood volume was normal (Bernardi et al, 1992a). The haemodynamic pattern "appears to be a function of the distribution of an expanded blood volume, trapped within the splanchnic area in the upright posture and redistributed towards the central and systemic circulations in the supine position" (Bernardi, 1993).

However, in advanced cirrhosis and ascites vasoconstrictor systems have been shown to be activated in the upright posture and not suppressed within the normal range by the supine-induced blood volume redistribution (Bernardi et al, 1992b). Although haemodynamics are a function of blood volume distribution, an activation of vasoconstrictor systems is also needed to

counteract a putative but very likely trend to arteriolar vasodilation. Therefore blood pressure homeostasis is achieved but at the price of renal sodium retention (Bernardi et al, 1992c). Thus the fundamental assumption of the peripheral vasodilation hypothesis is fulfilled, although it appears to mainly fit with what occurs in the advanced stages of cirrhosis.

THE COLLATERAL SYSTEM

When the portal circulation is obstructed, whether within or outside the liver, a collateral circulation develops to return portal blood into the systemic veins (Figure 3). In the normal liver all of the portal venous blood flow can be recovered from the hepatic veins but in cirrhosis only 10-15% is obtained; the rest enters collateral vessels. Blood from the gastro-oesophageal collaterals, retroperitoneal and venous systems of the abdomen ultimately reaches the superior vena cava via the azygos or hemiazygos system (Figure 3 & Appendix V). A small amount enters the inferior vena cava.

The presence of collaterals usually implies the existence of portal hypertension, although occasionally portal pressure can be normal if the collateral circulation is very extensive. The development of the collateral circulation reduces liver blood flow and the liver therefore depends increasingly on blood from the hepatic artery. It shrinks and its capacity to regenerate is impaired possibly due to a lack of hepatotrophic factors, including insulin and glucagon.

The major source of blood flowing through oesophageal varices is the left gastric vein, the posterior branch of which usually drains into the azygos system.

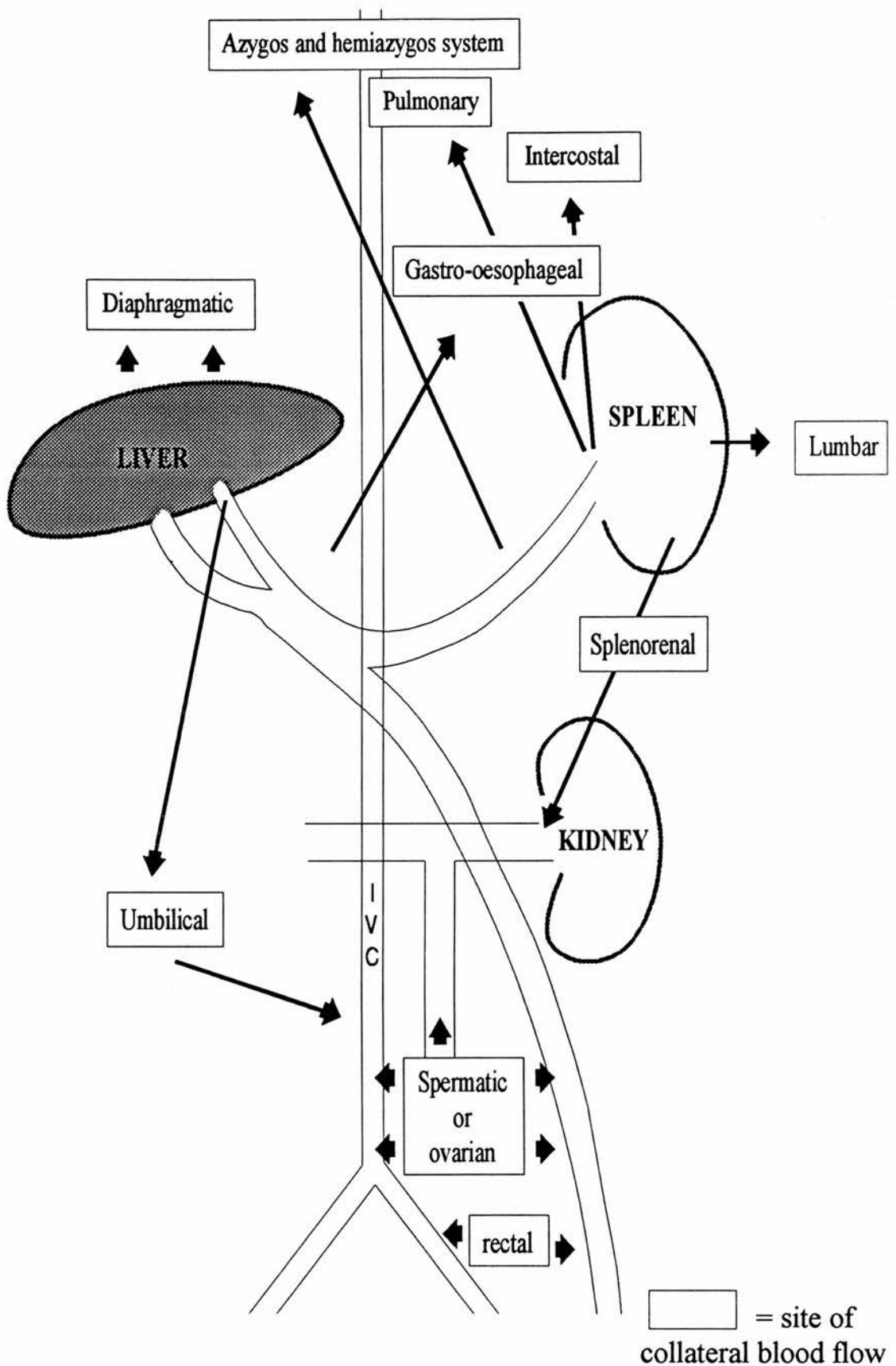


Figure 3. The collateral circulation

When the portal circulation is obstructed, a collateral circulation develops to return the portal blood into the systemic veins.

The anterior branch of the gastric vein communicates with varices just below the oesophageal junction and forms a bundle of thin parallel veins in the junction area and continue as large tortuous vessels in the lower oesophagus.

There are four layers of veins in the oesophagus (Figure 4). Intra-epithelial veins may represent the red spots seen on endoscopy (which predict variceal rupture). The superficial venous plexus drains into larger, deep intrinsic veins. Perforating veins connect the deeper veins with the adventitial plexus. Large varices arise from the main trunks of the deep intrinsic veins and these communicate with gastric varices.

The connection between portal and systemic circulation at the gastro-oesophageal junction is extremely complex (Vianna et al, 1987). A pallisade zone is seen between a gastric zone and the perforating zone. In the pallisade zone flow is bidirectional and this area acts as a watershed between portal and azygos systems. Turbulent flow in perforating veins between the varices and the perioesophageal veins at the lower end of the stomach may explain why rupture is frequent in this region (McCormack et al, 1983).

Recurrence of varices after endoscopic sclerotherapy may be related to the communications between the various channels or perhaps to enlargement of veins in the superficial venous plexus. Failure of sclerotherapy may also be due to failure to thrombose the perforating veins.

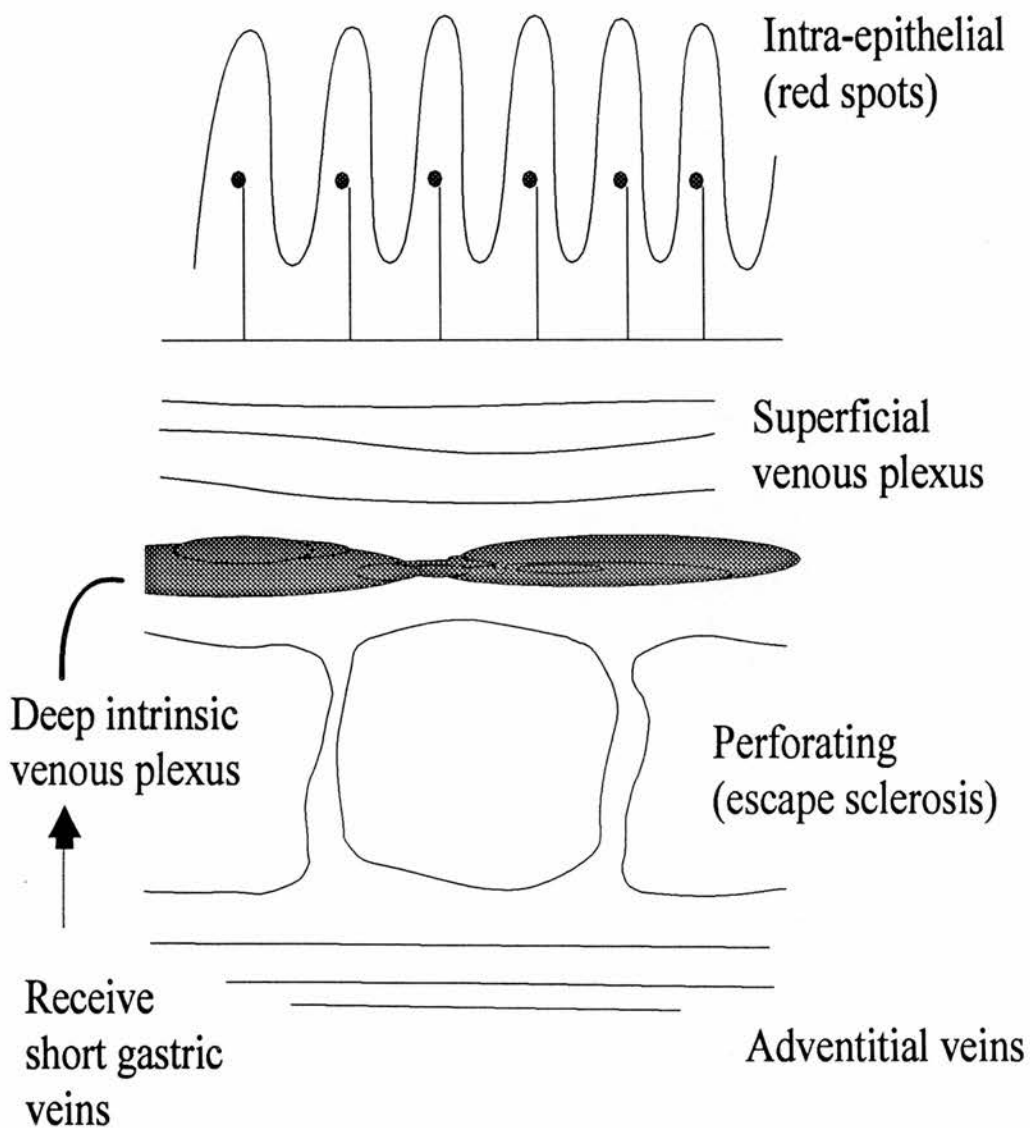


Figure 4

The layers of veins in the oesophagus.
(After Sherlock and Dooley, 1993)

PORTAL GASTROPATHY

In portal hypertension gastric vascularity is abnormal showing increased submucosal arteriovenous communications between the muscularis mucosa and dilated pre-capillaries and veins - a vascular ectasia (Quintero et al, 1987). This has been termed congestive gastropathy.

Such gastric mucosa may be at particular risk of bleeding and of damage, for instance, by aspirin or non-steroidal anti-inflammatory drugs. Bleeding can occur from gastric red spots (Quintero et al, 1987). These gastric changes may be increased after successful oesophageal sclerotherapy. They are relieved only by reducing the portal pressure.

CLINICAL FEATURES OF PORTAL HYPERTENSION

Haematemesis is the commonest presentation of portal hypertension although melaena, without haematemesis, may occur both from bleeding varices and portal hypertensive gastropathy.

Abdominal collateral veins

In intrahepatic portal hypertension, some blood from the left branch of the portal vein may be deviated via para-umbilical veins to the umbilicus and consequently to the caval system. The veins may be prominent over the anterior abdomen while in extra-hepatic portal obstruction, dilated collateral veins may appear in the flanks.

The direction of flow of the blood in these vessels is downwards. A number of prominent collateral veins radiating from the umbilicus is termed caput medusae but this is rare; the blood flow is away from the umbilicus. The presence of collateral vessels may also cause a venous hum (Cruveiler-Baumgarten murmur) which can sometimes be heard in the region of the xiphoid process or umbilicus with an associated thrill at the site of maximum intensity. It is due to blood rushing through a large umbilical or paraumbilical channel in the falciform ligament from the left branch of the portal vein to the superior epigastric, internal mammary or inferior epigastric veins. It is clinically significant in that it indicates a patent portal vein.

Splenomegaly

Splenomegaly is often associated with portal hypertension but its size bears little relation to the degree of portal pressure. The degree of splenomegaly tends to be larger in young patients and in those with macronodular cirrhosis (Sherlock and Dooley, 1993). Secondary hypersplenism may be associated with the splenomegaly of portal hypertension but is related to splenic size and hyperplasia of the reticulo-endothelial system rather than portal pressure.

Anorectal varices

These are found in 44% of cirrhotic patients and may cause significant blood loss (Hosking et al, 1989).

Oesophageal varices

Oesophageal varices show as protrusions into the lumen of the oesophagus on

endoscopy. Their size is graded (North Italian Endoscopic Club, 1988) as follows:

- Grade 1 - the varices can be depressed by insufflation of air through the endoscope.
- Grade 2 - the varices cannot be depressed by the endoscope.
- Grade 3 - the varices are confluent around the circumference of the oesophagus.

The larger the varix the more likely it is to bleed. Colour is also important.

Varices usually appear white and opaque. Redness correlates with blood flow through dilated sub-epithelial and communicating veins. Dilated sub-epithelial veins may appear as raised cherry-red spots and red wale markings. These colour changes predict variceal bleeding.

Bleeding is almost always localised to within the 5 cm above the cardia and this is the area in which sclerotherapy and band ligation is directed (Johnston, 1981).

Congestive or portal hypertensive gastropathy

This is seen mostly in the fundus, but in about one third of patients extends throughout the stomach. Sclerotherapy may increase gastropathy (D'Amico et al, 1990).

Ascites

Ascites is defined as a detectable collection of free fluid within the peritoneal space. Portal hypertension appears to be an important prerequisite for ascites

generation but ascites in cirrhosis always indicates liver cell failure in addition to portal hypertension (Sherlock and Dooley, 1993).

THE PATHOGENESIS OF ASCITES

Patients with ascites retain salt and water because homeostatic mechanisms become unbalanced (Arroyo et al, 1991). In the later stages of cirrhosis, there may also be absolute as well as relative reductions in renal blood flow and glomerular filtration rate due to vasoconstriction of the renal arteries. The exact sequence of events by which the diseased liver promotes renal salt and water retention and leads to renal failure however remains uncertain.

Hyperaldosteronism (which stimulates sodium reabsorption in the distal tubule of the kidney) and increased sympathetic nervous activity (which enhances sodium resorption in the proximal tubule, loop of Henle and distal tubule) both appear to be important factors in the pathogenesis of sodium retention in cirrhosis (Rosoff et al, 1975; Bichet et al, 1982a) and seem to outweigh the effects of endogenous natriuretic hormones (Gines et al, 1988; La Villa et al, 1990).

The kidney is also less able to excrete free water because of reduced delivery of filtrate to the ascending loop of Henle and hypersecretion of antidiuretic hormone (Bichet et al, 1982b; Perez-Ayuso et al, 1984). Finally, an imbalance between the activity of endogenous vasoconstrictor systems, for example the renin-angiotensin-aldosterone and sympathetic nervous systems, and the renal production of vasodilator substances, such as prostaglandins and renal

kallikrein, may account for the development of renal vasoconstriction and renal failure (Arroyo et al, 1986; Moore et al, 1990; Hayes et al, 1992b).

The degree of portal hypertension correlates well with the degree of stimulation of neurohormonal systems involved in the pathogenesis of salt and water retention and renal vasoconstriction (Bosch et al, 1980; Henriksen et al, 1984a). Consequently, any hypothesis concerning the pathogenesis of ascites needs to explain how portal hypertension might activate these systems.

Traditional theories of ascites formation

The **underfill theory** proposes that intrahepatic obstruction of blood flow increases sinusoidal pressure and thus lymph formation. The latter eventually exceeds the capacity for lymphatic return and accumulates in the peritoneal cavity as ascites (Greenway and Lautt, 1970; Witte et al, 1971; Henriksen and Ruig-Larsen, 1984b). As a consequence the intravascular plasma volume is reduced and intrathoracic and arterial mechanoreceptors activated, signalling the kidney to retain salt and water. Renal failure supervenes due to circulatory hypovolaemia.

According to this theory plasma volume and cardiac output should be low and peripheral resistance high in patients with cirrhosis. However, a hyperdynamic circulation is seen in patients with both compensated cirrhosis and with cirrhosis and ascites (Bosch et al, 1980), and in animal models of cirrhosis without ascites or prehepatic portal hypertension (Vorobioff et al, 1983 and 1984), thus this

theory does not seem to provide a plausible explanation, although it does explain the elevated catecholamine levels.

The **overflow theory** was postulated to explain the aetiology of ascites in the knowledge of the hyperdynamic circulation of cirrhosis (Lieberman et al, 1970). This theory holds that renal sodium retention is the primary event rather than a decrease in intravascular volume and is mediated via a hepatorenal reflex involving intrahepatic mechanoreceptors (Kostreva et al, 1980). As salt and water are retained, plasma volume expands, cardiac output increases and peripheral vascular resistance falls to accommodate the high blood volume. Higher pressure in the hepatic and splanchnic beds would result and lead to "overflow" ascites. Unfortunately, this theory does not explain the relative hypovolaemia, systemic hypotension and elevated catecholamines noted in cirrhotic patients.

Peripheral arterial vasodilation theory

Like the underfill theory this most recent theory, considers sodium retention as a secondary event with portal hypertension as the initial event. Splanchnic and, later, systemic arteriolar vasodilatation causes underfilling of the arterial vascular compartment, stimulates baro-receptors and subsequently of the renin-angiotensin-aldosterone system, sympathetic nervous system and antidiuretic hormone production leading to renal sodium and water retention.

In the early stages of cirrhosis when splanchnic vascular resistance is moderately reduced and the lymphatic system is able to return to the circulation the

increased amounts of hepatic and splanchnic lymph produced, arterial vascular underfilling is corrected by transient periods of sodium and water retention.

The fluid retained by the kidneys remains in the intravascular compartment, increases intravascular volume and cardiac output, refills the dilated arterial vascular bed, suppresses the signals that stimulate endogenous neurohormonal mechanisms, and normalises sodium and water excretion. With progression of liver disease however, splanchnic arteriolar vasodilation increases and simultaneously affects the general, hepatic and splanchnic microcirculations. In the general circulation, it accentuates arterial vascular underfilling while in the other two it causes more deterioration of the mechanisms that control transvascular fluid exchange. The higher splanchnic blood flow increases the hydrostatic pressure in the hepatic sinusoids and splanchnic capillaries. In addition, arteriolar vasodilation increases the filtration coefficient of the splanchnic microvascular barrier by affecting capillary permeability and the number of perfused capillaries (Granger and Barrowman, 1984). As a result along with hypoalbuminaemia, capillary hyperfiltration is enhanced and a critical level is reached at which the amount of fluid leaving the sinusoids and splanchnic capillaries exceeds the lymphatic return and the fluid accumulates in the intraperitoneal compartment. The renin-angiotensin-aldosterone system, sympathetic nervous system and secretion of antidiuretic hormone must then be continuously stimulated to maintain arterial pressure, which perpetuates sodium and water retention and the formation of ascites.

This hypothesis is compatible with all the haemodynamic changes observed to date in cirrhosis - only time will tell if it is the full story.

HAEMODYNAMIC ASSESSMENT OF THE PORTAL AND SYSTEMIC CIRCULATION AND THE ACTION OF DRUGS *IN VIVO*

The relationship between electrical current, electrical resistance and voltage is described by Ohm's law: $V = I R$.

When it is applied to a haemodynamic system, V is the pressure, I is the blood flow and R is the resistance. All components can be measured in an electrical system, but in haemodynamic studies, resistance cannot be measured and must be calculated indirectly from the measures of flow and pressure.

The effect of drugs on the haemodynamic system of humans may be evaluated if the limitations imposed on in vivo studies are accepted, which include the fact that isolation of individual parameters is often not possible and that measurements of the variables are sometimes indirect.

Methods for the assessment of portal pressure

Various methods for the measurement of portal pressure have been developed.

There are direct and indirect techniques:

a) INDIRECT METHODS

Hepatic vein catheterisation (i.e. wedged minus free hepatic pressure)

Intra-oesophageal variceal pressure

- direct puncture
- pressure gauge

Splenic pulp pressure

b) DIRECT METHODS

Transhepatic puncture (Chiba needle)

Umbilical vein catheterisation

The one most commonly used to evaluate the degree of portal hypertension *in vivo* is hepatic vein catheterisation with a balloon catheter.

Balloon catheterisation of the hepatic vein allows measurement of the portal pressure gradient with one procedure.

Myers and Taylor introduced the technique of hepatic vein catheterisation in 1951 and having been extensively used and modified since, it is generally regarded as safe, simple and capable of giving reproducible results. It can be performed in patients who have a bleeding tendency or ascites, but is of no value in patients with presinusoidal portal hypertension, in whom the wedged hepatic venous pressure (WHVP) is normal (Hoefs et al, 1990).

During this procedure the hepatic vein is occluded with a balloon-tipped catheter to create a stagnant vascular segment within the liver (Groszmann et

al, 1979). The catheter is known to be properly wedged if the pressure tracing shows tiny regular oscillations related to transmission of hepatic arterial pressure. When the balloon is deflated, the free hepatic vein pressure can be measured, which approximates to inferior vena cava pressure.

The difference between "wedged" (WHVP) and "free" (FHVP) pressure is the hepatic venous pressure gradient (HVPG) and is normally 5-6 mmHg. Values exceeding 10 mmHg indicate portal hypertension.

The WHVP closely reflects portal pressure in patients with alcoholic cirrhosis but is lower than portal pressure in patients with nonalcoholic causes of cirrhosis because of pre-sinusoidal factors (Boyer et al, 1977). During acute administration of vasoactive drugs, WHVP may not always provide a reliable measure of the absolute change in portal pressure but the direction of the change can adequately be assessed (Valla et al, 1984a).

Intra oesophageal variceal pressure (IOVP) can be determined either by direct puncture of a varix (Staritz et al, 1985) or indirectly using a pneumatic pressure gauge fixed to the tip of an endoscope (Mosimann, 1982). IOVP is lower than WHVP but correlates well with it (Rigau et al, 1989). It is not well suited for short term haemodynamic studies.

Splenic pulp pressure measurement (Atkinson and Sherlock, 1954) requires placement of a needle into the spleen and causes intra-splenic haemorrhage in about 1% of those studied. It therefore is not performed today except in the assessment of splenic or portal venous thrombosis.

Of the direct methods, transhepatic puncture of a portal vein-branch with a thin needle is unable to give prolonged measurements and is contra-indicated in patients with ascites. It is the technique of choice in the Budd-Chiari syndrome and in the assessment of patients with presinusoidal portal hypertension (Groszmann et al, 1979; Groszmann, 1984).

In portal hypertension, the umbilical vein is often patent and dilated, serving as a portasystemic collateral. It can be exposed by dissection of the pre-peritoneal fat cephalad to the umbilicus and a catheter can be manipulated through it into the left intrahepatic portal vein and ultimately the main portal vein to allow portal pressure measurement (Hoefs et al, 1990).

Direct methods of measurements of portal pressure have the limitation that inferior vena cava pressure is not measured. This is an important parameter to measure in an individual study as it provides the internal reference point and eliminates problems associated with poor reproducibility due to variations in the external zero point. Therefore, by measuring the inferior vena cava pressure as an internal reference point, an accurate assessment of any changes in the venous haemodynamic system may be described. This allows for comparisons of measurements made in different laboratories.

Assessment of hepatic blood flow

a) Indirect methods

- clearance technique
- inert gas washout

- angiography - radionuclides
- contrast dyes
- fractional distribution of microspheres
- indicator dilution technique

b) Direct methods

- doppler sonography
- electromagnetic flowmeter
- heat exchange
- timed collection of hepatic venous output

The oldest method for determination of hepatic blood flow is the clearance technique based on Fick's principle. Thus ELBF is the estimated liver blood flow,

$$\text{ELBF} = \frac{\text{IR}}{\text{Ca} - \text{Chv}}$$

where **IR** is the infusion rate of a highly extracted substance and **Ca** and **Chv** are the concentrations of this substance in the artery and hepatic veins, respectively. This method becomes unreliable when the extraction falls below 20% (Bradley et al, 1945) and it is therefore vitally important that extraction is determined hepatic vein catheterisation.

The ideal substance for estimation of liver blood flow has yet to be found but would have little extra hepatic metabolism and a constant volume of distribution. The extraction of taurocholate and indocyanine green (ICG) are

affected, to a similar extent, by liver cirrhosis (Paumgartner et al, 1979) while that of galactose seems to be slightly less affected (Henderson et al, 1982). ICG has the advantage of being removed only by the liver, its enterohepatic circulation is minimal, and it is therefore the substance most commonly used.

The inert gas washout method, although reliable and relatively non-invasive, is greatly limited in its application to man by the fact that the partition coefficient of the inert gas must be known, and is affected to different extents in different liver diseases; even within a single liver there may be regional differences.

In those patients where the diameter of the portal vein can accurately be estimated, calculation of portal flow is possible with Doppler ultrasonography i.e. duplex Doppler (Ohnishi et al, 1986). Satisfactory results depend on meticulous attention to detail and on technical expertise (Sabba et al, 1990). The method is most useful in measuring rapid, large, acute changes in flow rather than monitoring chronic changes in portal haemodynamics (Sabba et al, 1990, Alvarez et al, 1991).

Flows in exposed vessels may be measured directly using the square wave electromagnetic flow meter enabling those in the portal vein and hepatic artery to be measured separately.

The other methods listed above are either only semi-quantitative, for example angiographic methods, or too invasive for use in humans.

Assessment of portal-systemic collateral flow

Measurement of portal-systemic collateral flow is difficult.

The fraction of portal blood that is shunted may be computed by direct injection of isotope into the splenic or mesenteric vein and measurement of the area of an isotope dilution curve recorded in the hepatic vein.

Trans-hepatic catheterisation allows injection of isotopically labelled materials into the splenic or portal vein and differential counting over the liver and lungs allows calculations of intra- and extra-hepatic shunts. These values correlate well with the size of collaterals opacified by portography.

Most of the blood flowing through gastro-oesophageal varices drains into the azygos system. Measurement of blood flow in the azygos vein may therefore reflects blood flow in varices. Much of the flow is probably from para-oesophageal vessels rather than from endoscopically visible varices but since they are interconnected and have no valves, it is reasonable to assume that changes in azygos flow reflect changes in variceal blood flow.

Measuring azygos blood flow may help predict the risk of bleeding.

Propranolol, reduces the risk of bleeding and HVPG in only about 60% of patients but reduces azygos flow in all patients (Lebrec et al, 1981; Westaby et al, 1984; Mills et al, 1984; Bosch et al, 1984a; Cales et al, 1984).

Vasopressin and octreotide do likewise.

The original manual method described for measuring azygos blood flow employed a reverse thermodilution technique with calculations derived from a Wheatstone bridge and was cumbersome and time consuming, particularly when rapid changes in flow were recorded (Bosch and Groszmann, 1984b). Azygos blood flow can be now measured by a computerised system employing a continuous thermodilution technique (Hayes et al, 1992a) which allows real time measurement of the rapid changes in collateral blood which may occur with respiration, exercise, Valsalva manoeuvres etc. A catheter containing a calibrated thermistor (Webster Lab Inc) is placed under fluoroscopic control into the azygos vein, so that its tip is proximal to the arch of the vein. Flow in the vein can then be measured by the thermodilution principle using 5% dextrose at room temperature. The signals from the internal (injectate) and external (mixed blood) thermistors are transferred through a custom built interface and processed in an IBM model PS2-286 microcomputer to give real time values for blood flow.

The primary role of azygos blood flow studies are in the evaluation of new pharmacological therapies for portal hypertension.

Assessment of cardiac output

Cardiac output is defined as the volume of blood ejected by one ventricle in one minute: it equals the stroke volume multiplied by heart rate. In humans it can be measured by a variety of methods, which either measure cardiac output per se (the Fick principle and dilution methods) (Hamilton, 1962) or measure

stroke volume and heart rate separately (Doppler and radionuclide methods).

It can also be assessed indirectly by echocardiography.

i) Fick's principle

Adolf Fick observed in 1870 that the rate at which the circulation takes up oxygen from the lungs must equal the change in oxygen concentration in the pulmonary blood multiplied by the pulmonary blood flow. Since the pulmonary blood flow is the output of the right ventricle, this offered a way of determining cardiac output. In practice this only became possible in the 1940s, when progress in cardiac catheterisation allowed sampling of mixed venous blood to from the right ventricle. Peripheral venous samples are unsuitable because their oxygen content varies; venous blood only becomes fully mixed and uniform in the right ventricle outflow tract and pulmonary artery. The problem of obtaining mixed venous blood was first solved by the German physician Werner Forssman, who in 1929 passed a ureteric catheter through his own arm vein and into the heart, watching its progress on an X-ray screen. This act won him the disapproval of his head of department and, later, the Nobel prize.

The Fick method is currently as follows. The subject's resting oxygen consumption is measured over 5 to 10 minutes by collection of expired air in a Douglas bag. During this period an arterial blood sample is taken from the brachial, radial or femoral artery, and a mixed venous sample from the pulmonary artery or right ventricle outflow tract via cardiac catheter, introduced through the femoral or antecubital vein. The oxygen content of each blood sample is measured and the cardiac output (litres/min) calculated as:

$$\frac{\text{Oxygen uptake (mL/min)}}{\text{Arterial O}_2 \text{ conc.} - \text{Venous O}_2 \text{ conc.}}$$

While Fick's method is the yardstick by which new methods are judged, it has certain limitations. It is slow, and beat-to-beat changes in stroke volume cannot be followed. The method is only valid in the steady state, so that transient early responses to exercise or haemodynamically active drugs cannot be measured. It is also invasive and cannot be used during vigorous exercise because the cardiac catheter may provoke arrhythmias.

An indirect version, in which catheterisation is avoided and the O₂ content of mixed venous blood is estimated by analysis of rebreathed gas, is less accurate and rarely used today.

ii) Indicator dilution method

In Hamilton's indicator dilution method (1962), a known mass of an indicator is injected rapidly into a central vein or into the right heart. The indicator must be one that is confined to the bloodstream and easy to assay, for example indocyanine green, or albumin labelled with radioiodine. The bolus of indicator becomes diluted in the returning venous blood, passes through the heart and lungs and is ejected into the systemic arteries from which samples are taken at frequent intervals. The radial or femoral artery, is commonly used. The concentration of the indicator in the arterial plasma is measured and plotted against time. This gives the time (t) needed for a bolus to pass a given point and the mean concentration of indicator in the bolus over that period. Thus:

$$\text{Cardiac output of plasma} = \frac{\text{Mass of indicator}}{\text{mean concentration} \times \text{time } t}$$

The plot of concentration against time is a curve which rises to a peak and decays exponentially (Figure 5). The exponential decay is caused by the ventricle ejecting only a fraction of its content with each systole, leaving some indicator behind. Indicator-free venous blood returning to the heart during diastole dilutes the residual indicator, which in turn is only partially ejected in the next systole, and so on. After about 15 seconds the decay curve is disrupted by a "recirculation hump" (Figure 5) caused by blood with a high indicator concentration returning to the heart after completing one transit of the myocardial circulation (the shortest route back). To find the area under the concentration: time curve uncomplicated by recirculation, extrapolation is made of the early part of the decay curve, before the recirculation hump (for example by semi-log plotting and extrapolation to 1% of the peak value).

The results using this method agree to within 5% of Fick's direct method and has the advantages of improved time resolution (30 seconds versus more than 5 minutes for Fick's method) and ability to be used in exercise, since ventricular catheterisation is not required. The error involved in extrapolating the decay curve can be a serious limitation, particularly in patients with diseased hearts in whom the initial part of the decay curve may be short and distorted.



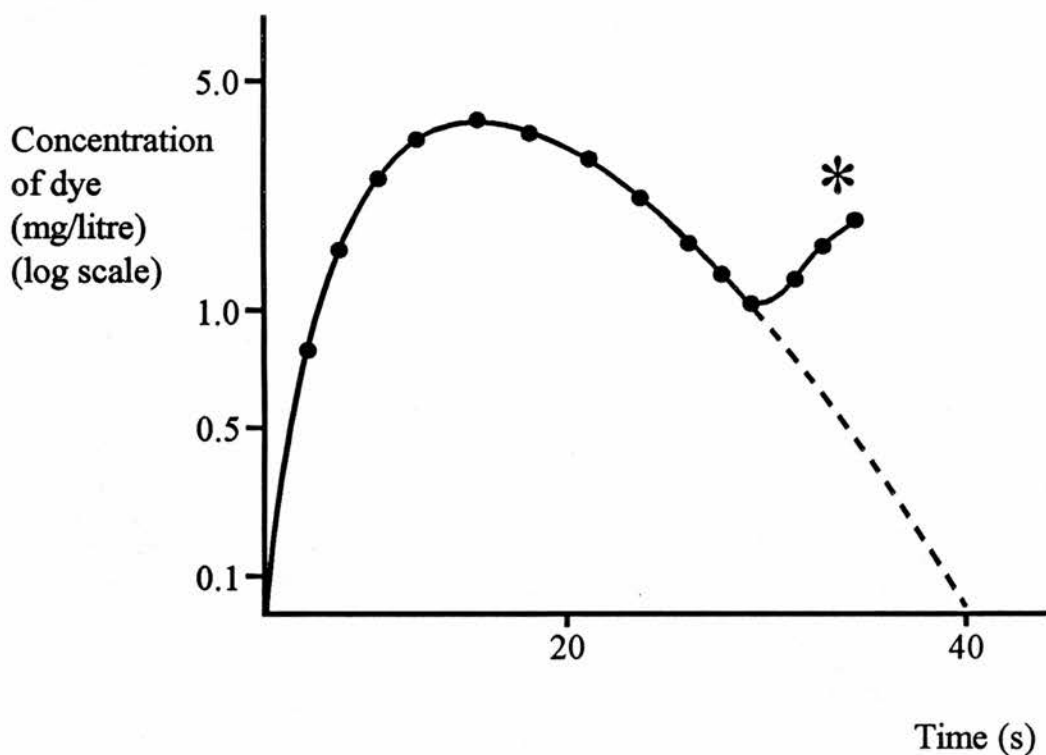


Figure 5

The concentration:time curve for Hamilton's dye dilution method for estimation of cardiac output

Concentration is plotted on a logarithmic scale to linearize the decay and allow extrapolation past the recirculation hump (asterisk).

Area under the extrapolated curve is used to calculate the cardiac output.

iii) Thermal dilution method

This is a variation of the dye dilution method above, is widely used in cardiac departments and is our method of choice for these studies.

A known volume of cold saline is injected quickly into the right atrium, right ventricle or pulmonary artery, and the dilution of the cold saline by warm blood is recorded by a thermistor-tipped catheter (Swan-Ganz catheter) in the more distal pulmonary artery. Cardiac output can then be calculated from the area under the temperature versus time plot and the amount of cold injectate.

The major advantage of this method is that the recirculation problem is circumvented because the saline warms up to body temperature long before it returns to the right side. Another is that the ejection fraction can be calculated from the step rise in temperature that follows each refilling of the heart by warm blood. One problem is that heat transfer across the walls of the right ventricle and pulmonary artery can cause over-estimation of the distribution volume and therefore cardiac output: a computed correction is usually made for this.

iv) Pulsed Doppler method (Loeppky et al, 1981; Tincani et al, 1993)

A pulse of ultrasound is directed down the ascending aorta from a transmitter at the suprasternal notch. Some of the ultrasound is reflected back by the red cells, and is collected. Since the cells have high velocity, the frequency of the returning sound waves is different from that of the transmitted signal; this is the Doppler effect.

The mean blood velocity across the aorta at each instant is computed from the spectrum of frequencies in the returning signal, and the velocity is plotted against time. To convert the time-averaged velocity (cm/s) to flow (cm^3/s) the diameter of the aorta must be measured by echocardiography and the cross sectional area multiplied by mean velocity. The result, aortic flow, represents cardiac output minus coronary blood flow. Doppler has calibration and "noise" problems, but is quick and non-invasive.

v) Other methods

Technetium 99m binds to red cells and when given intravenously allows estimation of cardiac output by counting over radioactivity the ventricles with a gamma camera. Such radionuclide angiography allows stroke volume and ejection fraction to be calculated from the difference between the radioactive content of the ventricles in diastole and in systole (Schelbert et al, 1978).

Echocardiography allows estimation of end-diastolic and end-systolic diameters of the ventricle which can be converted into stroke volume if some assumptions are made about chamber shape.

The electromagnetic flowmeter utilises a curved magnet with its poles directed on either side of the aorta or pulmonary artery. Since blood is an electrical conductor its flow induces an electrical potential when it intersects the magnetic field: the measured potential being proportional to velocity. The internal diameter of the vessel must be known to convert mean velocity to flow.

Use of the Swan-Ganz catheter

The development of the balloon tipped flow-directed catheter has greatly aided the precise measurements of cardiac output, pulmonary free and wedged pressure measurements and allowed sampling for oxygen utilisation studies. In our studies a triple lumen device was used. One lumen was used to inflate the balloon with air thus enabling the balloon to be carried by the blood flow through the right atrium and right ventricle into the pulmonary artery.

A second lumen opens at the tip of the catheter and is used for recording pressures and for withdrawing mixed venous blood.

A third lumen is positioned to open into the right atrium when the tip of the catheter is placed within the pulmonary artery. This permits the simultaneous determination of atrial and pulmonary capillary wedge pressure. A thermistor located approximately 4 cm from the catheter tip senses the temperature of the pulmonary arterial blood to permit determination of the cardiac output, as described previously.

Errors in estimation of mixed venous O₂ saturation and tension could occur if the blood is drawn rapidly or if the catheter is positioned peripherally within the pulmonary artery. These errors result from contamination of the mixed venous pulmonary blood with arterialised blood drawn from the pulmonary capillaries and veins.

The catheter frequently tends to migrate distally after insertion, causing the production of a continuous wedge pressure pattern with the balloon deflated.

The pressure must therefore be monitored continually so that the catheter may be repositioned immediately should a wedged trace appear. Pulmonary infarction could result from distal catheter migration.

The use of the Swan-Ganz catheter is not associated with high risk to the patient; however, the complications of pulmonary infarction, pulmonary artery rupture and air embolism are real.

PULMONARY SHUNTS IN CIRRHOSIS

Patients with cirrhosis show a wide range of arterial oxygen tension (Rodriguez-Roisin et al, 1992) despite normal pulmonary function tests. The pO_2 may be normal, yet as a result of hypocapnia there is an increase in the alveolar/arterial pO_2 difference. Alternatively some patients have mild-moderate arterial hypoxaemia and some may even need long-term oxygen therapy. The transfer factor for carbon monoxide may be normal or reduced.

Patients with cirrhosis have pulmonary shunting (Rodriguez-Roisin et al, 1992) but the exact form of such shunting has been highly controversial as emphasised by Berthelot et al, (1966):

" The tantalising problem of the connective link in cirrhotic patients between oxygen unsaturation and possible arteriovenous shunting in the lungs remains unsolved and any relation between arterial unsaturation and pulmonary vasodilation remains obscure".

Rydell and Hoffbauer (1956) were the first to identify at post-mortem numerous intrapulmonary arteriovenous anastomoses in a young patient with juvenile cirrhosis which were sufficient to cause cyanosis noted in life. The anastomoses

were seen between large arteries and veins near the hilum as well as in the peripheral vascular bed. Berthelot et al (1966) were the first to document post-mortem structural changes using a gold standard method of microgelatin injections into the vascular tree. Their finding was of dilation of the fine peripheral branches of the pulmonary artery at both the pre-capillary and capillary levels of the lung affecting arterial vessels up to 160 μ m in diameter and pleural spider naevi. Obvious arteriovenous communications as described by Rydell and Hoffbauer (1956) were found only in one patient however (Berthelot et al, 1966). Many studies have failed to identify precise anatomical pathways for intrapulmonary shunts and have postulated extra-pulmonary sites, for example, portal and pulmonary venous systems (Calabressi and Abelman, 1957; Rodman et al, 1959; Georg et al, 1960). All these studies however point to structural derangement of the pulmonary microcirculation sufficient to allow mixed venous blood to pass directly into the pulmonary veins in cirrhosis.

In addition physiological mechanisms almost certainly contribute to the hypoxaemia of cirrhosis. A right shift in the oxygen dissociation curve occurs in many patients (Rodriguez-Roisin et al, 1992) due to an increase in 2,3 diphosphoglycerate but is insufficient to explain the degree of hypoxaemia. The multiple gas elimination technique (Evans and Wagner, 1977) has shown that ventilation/perfusion mismatching may also be responsible in some patients. Patients with lower pulmonary vascular resistance appear to have a greater mismatch (Rodriguez-Roisin et al, 1992).

Therefore a wide range of gas exchange abnormalities may occur in patients with chronic liver disease. Whether the mechanism underlying some of these is related to failure of metabolism or of production of one of several circulating vasoactive substances by the damaged liver cells or to altered metabolism of paracrine factors synthesised by endothelial cells is unknown (Rodriguez-Roisin et al, 1992).

THE EFFECT OF INTRA-ABDOMINAL PRESSURE ON PORTAL PRESSURE, VARICEAL PRESSURE AND AZYGOS BLOOD FLOW

This concept is critical to an understanding of, for example, the role of ascites with respect to splanchnic haemodynamics and oesophageal variceal pressure.

If intra-abdominal pressure is increased by either ascites or air (for example, during laparoscopic liver biopsy), this pressure is transmitted to the intra-abdominal venous system (the muscular arterial walls limit pressure transmission to arteries).

Intra-abdominal pressure is usually identical to inferior vena cava pressure. Hence as intra-abdominal pressure increases, the IVC pressure rises in similar proportion, as does portal vein pressure, WHVP and FHVP. Thus, the portal pressure gradient and HVPG remain the same (Iwatsuki and Reynolds, 1973). With increased intra-abdominal pressure there are significant decreases in cardiac output and hepatic blood flow - whereas azygos blood flow increases markedly (Luca et al, 1993). Pressure within the oesophageal varices is not however increased in response to increased intra-abdominal pressure as the

varices are located downstream from the portal circulation. This may be explained by Figure 6. If a thin-walled tube is placed through a box in which the pressure can be varied and pressures are recorded at P1, P2 and P3, when the pressure in the box is increased, P1 and P2 increase since these are upstream from the site of resistance. P3 will not increase since it is downstream from the site of resistance. This concept is critical when interpreting the information derived from haemodynamic studies.

Therefore, ascites will increase the pressure within the portal system but not in the oesophageal varices. The increased abdominal pressure will be transmitted equally throughout the abdomen. It will not change the gradient between the sinusoidal pressure and the hepatic vein. When the intra-abdominal pressure is increased, the transmural pressure, a determinant of variceal rupture, is unchanged.

THE NATURAL HISTORY OF VARICES

The average risk of variceal bleeding for patients with cirrhosis and varices is 30%, with a 50% mortality rate within 6 weeks of the bleed (Burroughs et al, 1986). This mortality rate gives the rationale for prophylaxis to prevent the first variceal bleed.

Portal and intravariceal pressure (Burroughs et al, 1986), the appearance of endoscopically visible varices (Dagradi, 1972), severity of liver disease, including the presence of ascites (Burroughs et al, 1986) and alcohol abuse

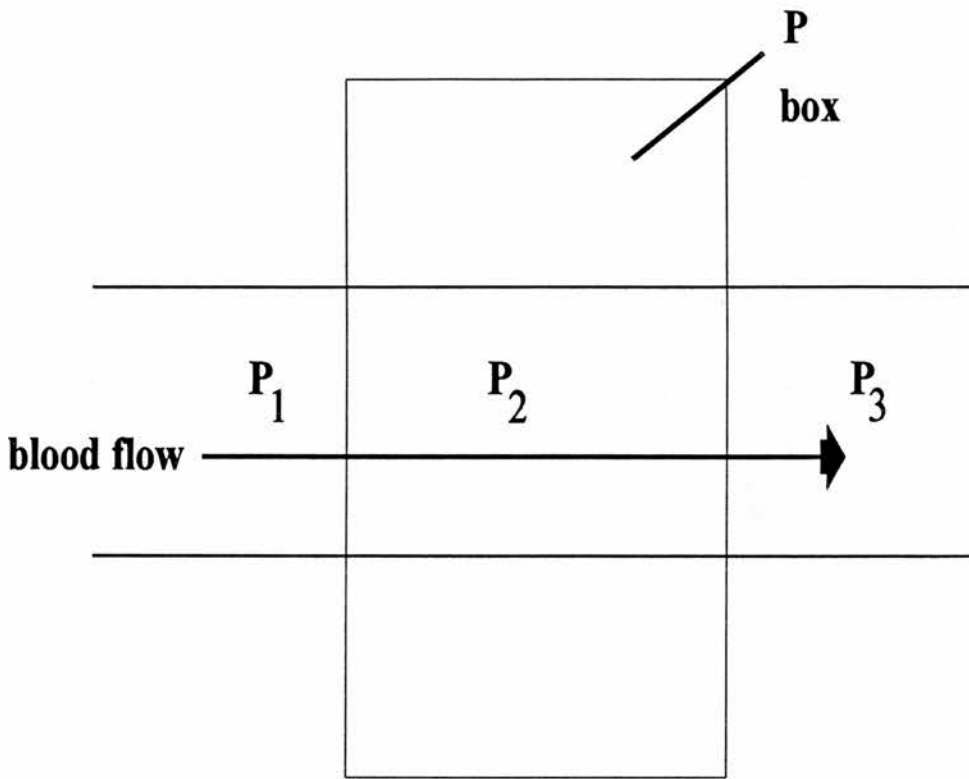


Figure 6

Schematic diagram of blood flow and pressure relationships to illustrate the effect of intra-abdominal pressure on portal pressure and variceal pressure

P₁, P₂ and P₃ represent separate sites of pressure measurement while pressure (P) in the box is variable.

(Dagradi, 1972) are independent risk factors for the occurrence of the first bleeding episode.

Although HVPG tends to be higher in patients who bleed or have large varices, bleeding risk is not related linearly to pressure above a threshold of 12 mmHg (Garcia-Tsao et al, 1985). Tension in the wall relative to varix radius may be critical and increasing variceal size, in conjunction with wall thinness, may favour rupture at lower intraluminal pressures (Polio and Groszmann, 1986; Kleber et al, 1991). The North Italian Endoscopic Club in 1988 formulated an index of risk for bleeding based on 3 independent risk factors; Child's-Pugh class, size of varices and presence of red wale markings on varices. This model has been validated by others and is the most useful prognostic index to date.

The justification for the use of prophylaxis in patients with their first variceal haemorrhage has been based on the well-documented high rate of recurrent bleeding that occurs in those patients who survive the first haemorrhage (Jones et al, 1973; Graham and Smith, 1981; Jensen and Krarup, 1989; Kahn et al, 1989). Over a two-year period this ranges between 50% and 100%, the variation largely reflecting different definitions of a rebleeding episode (Ollson, 1972; Westaby et al, 1985). The greatest risk of rebleeding occurs within the first 30 to 42 days of first presentation, a period that is associated with the highest mortality, and the most important factor predicting the incidence of rebleeding - the severity of underlying liver disease (Graham et al, 1981; Burroughs et al, 1986), with endoscopic findings such as variceal size and appearance being far

less significant; in contrast to their value at predicting first variceal haemorrhage (North Italian Endoscopy Club, 1988; Henderson et al, 1990).

The natural history of variceal bleeding, as described above, requires planned prophylaxis. The optimum treatment is one that is highly effective at preventing bleeding and is also applicable to patients with severe liver disease. Since 60-70% of patients with cirrhosis will never bleed from varices it is essential that prophylactic treatment is safe and free from side-effects.

NON-PHARMACOLOGICAL METHODS FOR THE PREVENTION OF VARICEAL BLEEDING OR REDUCTION OF THE HEPATIC VENOUS PRESSURE GRADIENT (HVPG)

TIPSS (Transjugular Intrahepatic Portasystemic Shunts)

This procedure was first described by Roesch and Hanafec in 1969. Portal decompression is achieved by percutaneously creating a channel between the hepatic vein and the portal vein with an expandable stent (Palmaz et al 1985; LaBorge et al, 1993). Its main use has been in the control of acute variceal bleeding (Zemel et al 1991, Ring et al 1992). Procedural problems are infrequent but serious life-threatening bleeding may occur. Long-term complications include encephalopathy and delayed shunt occlusion (Conn, 1993; Simpson et al 1993; McCaughan, 1994).

Surgical methods

Surgical options for prophylaxis of recurrent variceal bleeding include

decompressive shunts, devascularisation procedures such as splenectomy, oesophageal transection and oesophageal/gastric devascularisation and the ultimate surgical treatment of liver transplantation.

The use of total portal systemic shunts was popularised by Whipple in the 1940s but realisation of the cost of accelerated liver failure came in the next two decades (Jackson et al 1971, Resnick et al 1974, Reynolds et al 1981). The indications for this procedure are now limited to decompression the sinusoids of patients with acute Budd-Chiari syndrome and necrosis.

Various methods have however been used to perform partial portal systemic shunts. Selective shunts, such as the distal splenorenal shunt (DSRS), aim to compress the variceal system selectively and can reduce intravariceal pressure to less than 12 mmHg, but at the same time maintain portal flow to the cirrhotic liver. In studies comparing selective shunts and total shunts bleeding control and survival was found to be equivalent and encephalopathy was significantly lower, though not zero, in three of the six trials in the DSRS group (Conn et al 1981, Fischer et al 1981, Harley et al 1986, Langer et al 1985, Millikan et al 1985, Reichle et al 1979).

The rationale of devascularisation procedures, for example oesophageal transection, is that most varices bleed from the distal 5cm of oesophagus. However, this rationale might be questioned in light of data following chronic sclerotherapy that obliteration of varices at this high risk zone merely moves the bleeding sites to the gastric mucosa. In contrast to the shunt procedures in

which the primary risk is loss of portal flow and liver function, devascularisation procedures have rebleeding as their highest risk factor. Procedures range from very simple to very complex and the choice is determined by timing and the Child's-Pugh grading of the patient. Data to date suggest that whilst rebleeding is higher than with shunt procedures, liver failure is not being accelerated. Devascularisation procedures have had very variable success however (Idezuki Y et al 1990, Inokuchi et al 1985, Keagy et al 1986, Spence and Johnston, 1985).

The physiological goal of liver transplantation is to both relieve portal hypertension and restore a normal functioning hepatocyte mass. Rather than balancing the risk of bleeding against liver failure as with other surgical therapies, liver transplantation balances a normal liver and control of bleeding against immunosuppression. Interestingly, recent evidence suggests that prior variceal haemorrhage does not appear to adversely affect survival after liver transplantation (Ho et al, 1993). Ultimately whether to operate or not for variceal bleeding is based on the clinical pattern of variceal bleeding and the likelihood of survival. Not all patients with variceal bleeding could or should be transplanted.

Because superior mesenteric arterial blood flow is increased in portal hypertension and plays an important role in elevated portal pressure, mechanical reduction of artery diameter should decrease both portal pressure and superior mesenteric arterial blood flow. In rats superior mesenteric artery stenosis significantly reduced the degree of portal hypertension and the hyperdynamic circulation of rats with extrahepatic portal hypertension

(Soubrane et al, 1992). We can therefore speculate that superior mesenteric artery stenosis might provide a new therapeutic approach for portal hypertension in the future, though potentially at the risk of tissue ischaemia.

Endoscopic techniques

Endoscopic therapy (sclerotherapy or banding ligation) is the treatment of choice for control of acutely bleeding oesophageal varices. However its value in primary and secondary prophylaxis against that of pharmacological treatment has been the subject of much research. Sclerotherapy attempts to obliterate varices to prevent recurrent bleeding (Westaby and Williams, 1983). Injection may be intravariceal or paravariceal and in experienced hands the results of the two techniques appear similar. The number of sclerotherapy sessions necessary to achieve oesophageal ablation varies considerably which may be attributed to variations in the venous anatomy of the oesophagus (Westaby et al 1992). After variceal obliteration has occurred it is usual to follow patients at 3 month intervals for the first year and yearly thereafter.

A summary of the trials comparing beta-blocker with sclerotherapy in the primary prophylaxis and secondary prophylaxis of variceal haemorrhage is discussed below under beta-blockade.

Three studies examined the effects of adding propranolol to chronic sclerotherapy. In one study (Avgerinos et al, 1993), the combination was more effective at preventing bleeding from oesophageal varices, gastropathy and gastric varices than sclerotherapy alone. However, in the other two studies,

propranolol did not confer additional benefits (Acharya et al, 1993; Lo et al, 1993). Complications with sclerotherapy were more frequent and severe than with medical therapy and the technical requirements greater.

The most frequent complication of long-term injection sclerotherapy is due to oesophageal mucosal damage; ulceration at the site of previous injections is a very common finding (Terblanche 1983; Soderlund and Ihre 1985; Westaby et al 1985; Kitano et al 1988). However, recurrent bleeding due to mucosal ulceration occurred in almost 20% patients (Polson et al 1989). This does not appear to be affected by the type of sclerosant used but there is evidence from a large controlled trial that sucralfate reduces the frequency of rebleeding (Polson et al 1989). Deep ulceration may be associated with formation oesophageal strictures, a complication which occurs in about 10% of patients undergoing serial sclerotherapy. Omeprazole has been shown to be of value in the healing of the ulceration (Shepherd and Barkin, 1993). Oesophageal perforation has been reported in up to 5% of cases undergoing sclerotherapy, but the frequency has almost certainly been reduced since the introduction of fiberoptic techniques (Kahn et al 1989).

The incidence of pulmonary sequelae from sclerotherapy is high and includes effusions, infiltrates, atelectasis, and mediastinal enlargement; many are clinically silent. Serious pulmonary problems, including the adult respiratory distress syndrome, have however been reported in association with sclerotherapy and bacteraemia may also occur in 5-10% of patients. Other common effects include chest pain which lasts from 24-48 hours in up to 40% of

patients. Long term sequelae of sclerotherapy may include effects on oesophageal motility.

Six prospective trials compared sclerotherapy with shunt surgery. One was restricted to high risk actively bleeding patients and demonstrated that sclerotherapy was not superior to portocaval shunt placement. The remaining trials compared stable patients initially treated by sclerotherapy with those initially treated with portocaval or selective splenorenal shunt operations. Patients who failed sclerotherapy had salvage shunt operations. The Emory trial showed that survival and preservation of hepatic function were superior in patients initially treated with sclerotherapy, in spite of the fact that 35% of sclerotherapy patients eventually required a shunt operation (Millikan et al 1985). The Nebraska study showed that patients who were non-compliant or lived long distances from the treatment centre were better treated with a selective shunt but the other trials showed no differences between treatments.

Two other local endoscopic measures have been developed with the aim of overcoming some of the limitations of sclerosing agents. One involves the direct injection of the tissue adhesive cyanoacrylate (Histacryl) directly into the varices, with the aim of immediate luminal occlusion, without widespread mucosal damage (Soehendra et al 1986). This technique has been most widely adopted in the management of active variceal haemorrhage, particularly when this originates from fundal gastric varices. This technique is also currently being evaluated for long-term repeated injection.

Banding ligation is performed using a ligating device which attaches to a flexible gastroscope. Selected oesophageal varices are aspirated into the device and ligation is accomplished by small elastic rings. Strangulation of the tissue appears to take place with subsequent thrombosis of the submucosal varix (Marks et al, 1993).

Banding (Van Steigman and Goff 1988) can eradicate oesophageal varices with a median of 5 sessions, with recurrent acute variceal haemorrhage occurring in 47% of cases so treated (Van Steigman et al 1989). The technique is very safe with no reported incidence of oesophageal perforation.

Trials of variceal band ligation versus sclerotherapy in acute bleeding and the prevention of rebleeding are now beginning to be published (Stiegmann et al, 1992; Laine et al, 1993; Gimson et al, 1993). Gimson et al (1993) reported reduced incidence of rebleeding with banding, Laine et al (1993) reported fewer complications and Stiegmann et al (1992) reported increased survival in the banding group.

As stated above endoscopic ligation appears to eradicate varices with fewer treatments than sclerotherapy, a finding which may in part explain the lower incidence of recurrent bleeding observed in ligation treated patients and may result in an economic advantage for the new method. Prophylactic banding has also been reported, though has not yet been compared with medical therapy in a randomised controlled setting (Hashizumo et al, 1993).

Successful treatment of bleeding from oesophageal varices and prevention of recurrent bleeding remains a difficult problem. A number of medical, surgical, radiological and endoscopic treatment options are available. The choice of treatment should be based on local experience of methods and individual patient considerations such as compliance, proximity to treatment centre and whether the patient would be a candidate for eventual liver transplantation.

However, pharmacological agents have the advantage of being the easiest to administer.

THE PHARMACOLOGY AND HAEMODYNAMIC EFFECT OF DRUGS ON THE PORTAL AND SYSTEMIC CIRCULATION AND THEIR USE IN TREATMENT AND PROPHYLAXIS OF VARICEAL BLEEDING

Interest in the pharmacological therapy of portal hypertension has recently gained momentum because of the introduction of new drugs into clinical practice and their efficacy in prophylaxis and treatment of variceal bleeding. However, no perfect drug has been found to date and much remains to be discovered about the best dosing regimens for the drugs which are currently available.

The drug treatment of portal hypertension is based on the assumption that a sustained reduction in portal pressure reduces the incidence of complications. Studies have shown that when HVPG is reduced below the threshold of 12 mmHg, the patient is no longer at risk for variceal haemorrhage, and survival is

increased (Groszmann et al, 1990). This appears to be because of reduced variceal wall tension, which is directly related to variceal pressure when even in patients in whom the HVPG falls but remains more than 12mmHg, the risk of bleeding is reduced the tension exceeds a critical value the wall ruptures (Polio and Groszmann, 1986; Rigau et al, 1989). For example propranolol reduces the risk of bleeding but does not reduce the HVPG in 40% of patients (Lebrec et al 1982).

Pharmacological agents can be divided into vasoconstrictors (vasopressin, somatostatin, beta-blockers) and vasodilators (nitroglycerin, isosorbide mono or dinitrate, molsidomine, ketanserin). The vasoconstrictors cause splanchnic arterial vasoconstriction which leads to reduction of portal blood flow and pressure. The vasodilators can also lower portal blood flow and pressure by producing a peripheral vasodilation which causes reflex splanchnic vasoconstriction and a reduction in portal venous blood flow. Vasodilators also reduce intrahepatic resistance and importantly, dilate the porto-collateral circulation. The use of vasodilators, such as nitrates, to reduce portal hypertension has attracted a lot of research interest over the past few years as they have a theoretical advantage over vasoconstrictors, such as beta-blockers, in that they may allow reduction of portal pressure without impairment of liver perfusion (Navasa et al, 1989).

PHARMACOLOGICAL AGENTS OTHER THAN NITRATES SHOWN TO ACHIEVE REDUCTION OF THE HEPATIC VENOUS PRESSURE GRADIENT OR PREVENT BLEEDING FROM VARICES

Beta-Blockers

Lebrec was the first to report the beneficial effect of beta-blockade on variceal bleeding. Acute or chronic administration of beta-blockers to patients with portal hypertension has been shown to induce acute or chronic reduction in HVPG and azygos blood flow (Lebrec et al 1982; Cales et al 1984; Groszmann et al, 1990; Weinshel et al, 1994). This effect, when using non-selective beta-blockers (such as propranolol), appears to be due to a decrease in cardiac output as well as other factors such as vasoconstriction in the portal territory due to unopposed alpha-vasoconstriction (Hillon et al 1982).

The changes in HVPG do not appear to be greatly affected by the dose of beta-blocker used; however, the response varies widely from one patient to another. Propranolol affects HVPG less than WHVP (Valla et al 1984a) and has no effect in some cirrhotic patients (Bosch et al 1984a, Braillon et al 1986, Garcia-Tsao 1986). Even in patients showing no WHVP response, azygos blood flow is decreased (Braillon et al 1986, Garcia-Tsao 1986). This suggests, as with organic nitrates (discussed below) that beta-blocking agents may have differential effects on the portal and azygos systems.

The haemodynamic action of propranolol in patients with Child's Class heterogenous and controversial. Down-regulation of beta-receptors has been

described in cirrhotic patients with ascites (Gerbes et al 1986) and may explain the heterogeneous response in such patients.

Propranolol is a beta-blocker with both beta 1- and beta 2- blocking characteristics i.e a non-selective beta-blocker. Nadolol is a less lipophilic non-selective beta-antagonist which is eliminated by renal excretion, does not cross the blood brain barrier and therefore may be less likely to cause central nervous system effects, although this has yet to be proven in patients with cirrhosis. It has a long half-life allowing once daily administration. Both nadolol and propranolol are accepted therapeutic agents for the prevention of variceal bleeding or rebleeding (Pagliaro et al 1989, Poynard et al 1991a, Conn et al 1991, Ideo et al 1988, Lebrec et al 1988).

A certain degree of beta 2- antagonism appears to be needed to achieve portal pressure reduction; thus metoprolol, a selective beta 1- antagonist, reduced WHVP only to the extent predicted by its reduction in cardiac output but mepindolol, an agent with predominant beta 2- antagonism, reduced WHVP to a greater degree. In contrast to propranolol however it markedly reduced hepatic blood flow (Braillon et al 1985). No evaluation has therefore been made of these selective drugs in long-term, randomized, controlled clinical trials.

The merits of the different beta-blocking agents in the prevention of oesophageal variceal bleeding have been reviewed (Ohara et al 1986, Groszmann et al 1985, Bosch 1985, Hayes et al 1990, Pagliaro et al 1992). From

these reviews it would appear that both beta 1- and beta 2- antagonism are required for an agent to be clinically useful.

PREVENTION OF FIRST VARICEAL HAEMORRHAGE

There are to date 9 placebo-controlled studies of the prevention of first variceal bleeding (Pascal et al, 1987; Colman et al, 1988; Strauss et al, 1988; Lebrec et al, 1988; Ideo et al, 1988; The Italian Multicenter Project for propranolol in prevention of bleeding, 1989; Andreani et al, 1990; Conn et al, 1991; the PROVA study group, 1991).

In four of these studies, all the patients had large varices; in the remaining 5 studies patients with either small or large varices were included. Follow up was for a period of 1 year in 2 studies and 2 years in the other studies. 7 studies used propranolol or Inderal (LA) at doses that decreased heart rate by approximately 25% (40-300 mg/day). In the other 2 studies nadolol was used instead (Ideo et al, 1988; Lebrec et al, 1988). In patients who received placebo, the incidence of bleeding reported ranged from 12% to 30% at 1 year and from 5 to 61% at 2 years. The incidence of bleeding in the beta-blocker group ranged from 0 to 18% at 1 year and from 6 to 26% at 2 years. 4 studies showed that beta-blocker use significantly decreased the incidence of initial gastrointestinal bleeding (Pascal et al, 1987; Ideo et al, 1988; Andreani et al, 1990; Conn et al, 1991). One other study observed this difference only among compliant patients (Lebrec et al, 1988) and another only when patients without ascites were considered (The Italian Multicenter Project, 1989). Of great importance was

that only one study showed a significant difference in survival rate at 2 years between patients who received propranolol and those who received placebo (Pascal et al, 1987).

Three published meta-analyses (Pagliaro et al, 1989; Hayes et al, 1990; Poynard et al, 1991a) conclude that the use of beta-blockers significantly decreased the risk of initial gastrointestinal bleeding in patients with cirrhosis and varices. Fatal bleeding was also significantly less frequent in patients who received beta-blockers than in those who received placebo (Poynard et al, 1991a).

When patients were tapered off their propranolol after the 2 years of study follow-up observation was continued by the Boston-New Haven-Barcelona Liver Group who found that the risk of bleeding recurred in these patients. The implication was therefore of a need for prolonged beta-blocker therapy to prevent initial variceal haemorrhage, a conclusion that needs verification in a long-term prospective trial.

Side-effects occurred in 3-40% of patients who received beta-blockers and use had to be discontinued for this reason in 5% of the patients (Poynard et al, 1991b). Mild and transient encephalopathy (1.4%) was observed in some studies. Perhaps surprisingly, in all trials resuscitation after bleeding was not more difficult in patients who received beta-blockers than those who did not.

The efficacy of beta-blockers and endoscopic sclerotherapy in prevention of the first episode of gastrointestinal bleeding were compared in 2 controlled clinical trials (Andreani et al, 1990; The PROVA Study Group 1991). Andreani et al

(1990) showed the risk of bleeding was significantly lower in patients with endoscopic sclerotherapy, whilst the PROVA group showed no statistical difference; neither showed a significant difference in survival between the two treatment groups.

PREVENTION OF RECURRENT VARICEAL HAEMORRHAGE

As with the trials for prevention of first variceal haemorrhage, only nonselective beta-blockers have been evaluated for the prevention of rebleeding from oesophageal varices. The time interval between haemorrhage and inclusion in the studies described below ranged from 1 day to more than 1 month.

Eleven randomized, controlled trials with follow up over 1 or 2 years have shown that nonselective beta-blockers were effective in preventing recurrent variceal bleeding with a reduction in risk of rebleeding from 65.9% in the control groups to 44.7% in the beta-blocker treated patients ($p < 0.0001$) (Burroughs et al, 1983; Lebrec et al, 1984; Cerbelaud et al, 1986; Villeneuve et al, 1986; Gatta et al, 1987; Queuniet et al, 1987; Colman et al, 1988; Colombo et al, 1989; Sheen et al, 1989; Garden et al, 1990; Rossi et al, 1991).

The risk of rebleeding differed widely among studies in both placebo and beta-blocker groups. In the placebo groups the risk of rebleeding at 1 year ranged from 10-71% and at 2 years from 21-72%. 6 studies found the risk of recurrent bleeding was significantly lower in patients on beta-blockers than placebo; 5 studies showed no difference. In only one study was a significant difference in survival observed at 2 years (Lebrec et al, 1984).

Two meta-analyses confirmed that beta-blocker use significantly reduced the risk (by about 20%) of recurrent gastrointestinal bleeding in patients with portal hypertension (Pagliaro et al, 1989; Hayes et al, 1990).

9 studies compared the efficacy of beta-blocker use with that of endoscopic sclerotherapy in the prevention of recurrence of gastrointestinal bleeding in patients with cirrhosis (Fleig et al, 1987; Alexandrino et al, 1988; Dollet et al, 1988; Westaby et al, 1990; Qureshi et al, 1990; Andreani et al, 1990; Rossi et al, 1991; Martin et al, 1991; Teres et al, 1993). Six randomized, controlled trials have found no significant difference between the two groups, two studies found that the risk of rebleeding was lower in patients who received beta-blockers than in those treated with endoscopic sclerotherapy (Alexandrino et al 1988; Andreani et al, 1990) and one study found that patients treated with sclerotherapy had a significantly lower incidence of rebleeding than patients who received beta-blockers (Westaby et al, 1990).

It has been shown that the combination of endoscopic sclerotherapy with beta-blockade is more effective in preventing recurrent gastrointestinal bleeding than either form of therapy alone (Jensen and Kuarup, 1989; O'Connor et al, 1989; Vinel et al, 1990; Ink et al, 1990).

Contra-indications and side-effects of beta-blockers

The limitations of therapy with non-selective beta-blockers are related to their contraindications and side effects. Contraindications include chronic obstructive airways disease, asthma, congestive heart failure, atrio-ventricular heart block,

arrhythmias and psychosis (Conn et al 1991). Side effects occur in up to 15% of patients, but serious events such as bronchospasm are rare (Conn et al 1991). The more frequent complaints are fatigue and sleep disorders. Side effects are however extremely important as they alter compliance.

As 30-40% of patients either have contraindications to the use of nonselective beta-blockers or develop side-effects requiring cessation of treatment, alternative pharmacological agents are needed.

Alpha-adrenergic agents

Patients with cirrhosis often show signs of sympathetic nervous system overactivity with increased plasma noradrenaline levels. Thus it was reasoned that clonidine, a centrally acting alpha-2-agonist could be used to reduce these, and may also reduce portal pressure.

Two studies showed that clonidine decreased plasma noradrenaline and the HVPG (Willett et al 1986, Moreau et al 1987). In both, clonidine significantly reduced cardiac output and mean arterial pressure. The mechanism of portal pressure reduction may be due to an affect on post-sinusoidal hepatic vascular resistance (Willett et al 1986) as well as reduction of cardiac output inducing splanchnic vasoconstriction (Moreau et al 1987).

Arterial pressure is markedly reduced with clonidine but this does not appear to affect renal function (Debinski et al 1989) or hepatic function as assessed by hepatic blood flow, galactose-elimination capacity, hepatic clearance of indocyanine green and hepatic intrinsic clearance of indocyanine green

(Albillos et al, 1992b). In none of the patients given clonidine for a mean period of 64 ± 10 days was the drug withdrawn because of side-effects, although 12 subjects complained of dry mouths (Albillos et al, 1992b).

Phentolamine and prazosin are also alpha-blocking agents which can reduce portal pressure in man (Mills et al 1981). Prazosin, in contrast to beta-blocking agents, decreases WHVP without affecting cardiac output (Mills et al 1981).

However, although the alpha-adrenergic antagonists described above are effective portal hypotensive agents, their systemic hypotensive effect may preclude use in the already vasodilated cirrhotic subject.

Calcium antagonists

Verapamil was the first such agent to undergo study and rat studies demonstrated a portal hypotensive action (Reichen and Lee, 1986). Subsequent human studies were however disappointing (Navasa et al, 1988; Vinel et al, 1989). Nifedipine was shown to be ineffective at portal pressure reduction (Lay et al, 1987) and possibly harmful (Koshy et al, 1987).

Thus calcium channel blockers, although theoretically likely to be useful have no role to play in the treatment of portal hypertension.

Serotonin S₂ receptor blockers

Ritanserlin and ketanserlin have been shown to lower portal-collateral resistance in animal models of portal hypertension and in patients with cirrhosis (Mastai et al 1990, Nevens et al 1991, Hadengue et al 1987, Vorobioff et al 1989,

Fernandez et al, 1993). These studies show that selective S₂-receptor antagonists do not cause a decrease in arterial pressure and the potential clinical use for these drugs requires further evaluation.

Other drugs

Whether **angiotensin-2-antagonists** or angiotensin-converting enzyme inhibitors have any role in the pharmacotherapy of portal hypertension remains dubious. Captopril, an angiotensin-converting enzyme inhibitor, lowers WHVP in patients with cirrhosis (Eriksson et al 1984); this is due mainly to induction of systemic hypotension (Pariente et al 1985).

Spironolactone, an aldosterone antagonist, lowers HVPG in patients with cirrhosis without ascites and reduces plasma volume, which reduces cardiac output and thus triggers vasoactive mechanisms that decrease splanchnic blood flow (Okumura et al 1991, Garcia-Pagan JC et al 1994, Katsuta et al, 1993). Potentially, spironolactone may maintain and enhance the decrease in portal pressure achieved by nitrates or propranolol as plasma volume contraction may logically be expected to relieve portal hypertension. **Furosemide** however does not reduce HVPG (Katsuta et al 1993).

Molsidomine (SIN-10), a selective reducer of pre-load with nitrate-like effects but a longer duration of action is not associated with a significant reduction in blood pressure or development of tolerance (Bassenge, 1982). Vasoactive SIN-1A is formed only after metabolism of molsidomine to SIN-1 in the liver

although no delay in the onset of action has been reported in patients with chronic liver disease (Bhome, 1990).

Single doses of molsidomine (eg 2 or 4 mg) given intravenously significantly reduced HVPG without clinically relevant impairment of systemic cardiovascular regulation (Huppe et al, 1992). The reduction in HVPG resulted from vasodilatation in the venous circulation and a reduction in cardiac output, with a simultaneous slight reduction in MABP. However, there was no correlation between MABP, cardiac output reduction and the fall in HVPG. Since SVRI also showed no significant changes, it is assumed that the effect of molsidomine in reducing HVPG can be mainly attributed to vasodilatation and a decrease in pressure in the splanchnic region, probably associated with a decrease in portal resistance (Kukovetz and Holzmann, 1985). However in the study of Huppe et al (1992), the overall significant reduction in HVPG masked the fact that 8 patients were non-responders; in 3 there was no change in HVPG, while in 5, the HVPG actually rose. In general, the non-responders were characterised by a greater reduction in FHVP than WHVP, and marked ascites.

Similar results have been published elsewhere; Vinel et al (1990) showed a 15.4% reduction in HVPG 60 minutes after oral administration of 4 mg molsidomine with a non-responder rate of 23.1%. Ruiz del Arbol et al (1991) showed a dose dependent reduction in HVPG of 10.7% and 19.2% after 120 minutes. Both groups showed that a significant reduction in hepatic blood flow was associated with a decrease in HVPG, without any impairment of hepatic

clearance function. Whether this drug will be effective at prophylaxis of variceal haemorrhage remains to be established by clinical trials.

THE PHARMACOLOGY AND HAEMODYNAMIC EFFECT OF NITRATES ON THE PORTAL AND SYSTEMIC CIRCULATION AND THEIR USE IN TREATMENT AND PROPHYLAXIS OF VARICEAL BLEEDING (1^o and 2^o)

Isosorbide dinitrate and isosorbide-5-mononitrate (Is-5-Mn) are both "long-acting" organic nitrates. The mononitrate is the active component and is formed from the dinitrate by rapid denitration in the liver (Down et al, 1974; Spörl-Radun et al, 1980; Abshagen et al, 1985).

Is-5-Mn is a powerful venous and mild arterial dilator, with dose-dependent activity (Bogaert and Rosseel, 1972) and little first pass metabolism (Abshagen et al, 1981; Abshagen et al, 1992). It has a prolonged half-life (5 hours) (Abshagen et al, 1981; Taylor et al, 1981; Abshagen et al, 1992) and dose linear kinetics (Abshagen, 1986) even in the presence of liver disease (Steudel et al, 1983; Akpan et al, 1985) or renal failure (Raue et al, 1985).

In contrast, the dinitrate has a high first pass effect with an apparent oral bioavailability of 0.2 in normal subjects (Fung, 1985). This increases up to a mean of 0.65 in cirrhosis because of reduced hepatic extraction and the presence of portasystemic shunts (Blei et al, 1987). As these show inter-individual variation, the appropriate dosage of the dinitrate is difficult to determine and the response is unpredictable in patients with chronic liver

disease (Blei, 1986a). Such reasons make Is-5-Mn preferable to other organic nitrates in patients with cirrhosis.

Nitroglycerin (GTN) is a short acting nitrate with no theoretical advantages over Is-5-Mn unless oral administration is contraindicated, for example, eg acute variceal bleeding.

Adverse reactions associated with nitrates are unusual other than a mild headache resolving within 24 hours of the first dose occurring in up to one third of patients (Blei et al, 1987).

The molecular mechanism of nitrate action is controversial. In 1977 two groups independently demonstrated that organic nitrates induced a dose-dependent increase in the levels of cyclic guanosine-monophosphate in smooth muscle cells (Schultz et al, 1977; Katsuki et al, 1977). Subsequently it was shown that many other nitrovasodilators, including nitric oxide, activate soluble guanylate cyclase (Murad et al, 1978; Kukovetz et al, 1979).

Some vasodilators have been shown to generate nitric oxide in a nonenzymic reaction with cysteine (Moncada et al, 1988). Nitrates do not directly stimulate nitric oxide synthetase as demonstrated by Vallance et al (1989) who showed that GTN infusion at 2 and 5 nmol/min to human controls caused a dose-dependent increase in forearm blood flow which was unaltered by L-N-monomethyl-arginine administration (L-NMMA), a specific inhibitor of nitric oxide synthetase. It therefore seems likely that nitrates produce an increase in cyclic-GMP levels in smooth muscle by direct action rather than via

nitric oxide synthetase. There is strong evidence for thiol intermediates as modulators of the cellular events of nitrates (Horowitz et al, 1983).

Acute effects of organic nitrates on portal and systemic haemodynamics - animal studies

Vatner and colleagues (1978), demonstrated that intravenous nitroglycerin administration to normal dogs resulted in a significant decrease in mesenteric blood flow after an initial increase. The fall in mesenteric blood flow was associated with a reduction in arterial pressure and an increase in heart rate and it was suggested that nitrate-induced venodilatation resulted in baroreceptor-mediated mesenteric arterial vasoconstriction, a sympathetic response to vasodilation and venous pooling (a reduction in preload accounting for the reduced cardiac output).

In the portal vein ligated rat (an experimental model of portal hypertension) splanchnic vasoconstriction has been demonstrated after nitrate administration (Blei et al, 1984; Kroeger and Groszmann, 1985; Blei and Gottstein, 1986b).

Other studies have demonstrated that nitrates may reduce portal pressure by more than one mechanism, for example, myofibroblasts have been demonstrated in a perisinusoidal location in the cirrhotic liver and may be involved in the increased resistance to portal flow (Bhatal and Grossman, 1982). In the isolated perfused cirrhotic rat liver, administration of nitrates decreased intrahepatic resistance (Bhatal and Grossman, 1985). Nitrates may also have a direct vasodilatory effect on the collateral circulation: high dose nitrates given

Table 4

THE ACUTE PORTAL AN SYSTEMIC HAEMODYNAMIC EFFECT OF NITROGLYCERIN ADMINISTRATION TO PATIENTS WITH CIRRHOSIS

	Staritz, 1985	Garcia-Tsao, 1987	Westaby, 1988	Moreau, 1989	Rector, 1990	Twao, 1991
No of patients	14	11	12	11	10	14
Dose, route	(1.2 mg spray onto tongue)	(0.6 mg SL 2-12 mins peak range)	(200 ug/min i.v. peak change)	(7-15 ug/min i.v. peak change)	(40 ug/min i.v. peak change)	(Transdermal tape 10 mg)
Time		2-12 mins peak range				60 mins
SVRI			1887-1784	1537-1581		
WHVP		22.5-18.9*	28.7-25.8*	29.2-29.3	28-27	26.0-22.1*
HVPG		17.9-15.1*	16.4-13.3*	20.9-21.1	16-16	17.9-15.1*
AzBF		604-437		0.53-0.55	0.46-0.46	
ELBF				1340-1100		925-919
HR		73-83*	84-97	82-89.5*	84-87	72-71
MABP		96-76*	95-79*	86.8-79.4*	89-74*	106.1-91.3*
CO(a)/CI(b)			4.11-3.29* (b)	4.29-3.9* (b)	7.0-6.3* (a)	
IOVP Grade III varices	22.8-12.0*					
IOVP Grade II varices	16.3-10.0*					

KEY: * denotes significant difference at least at 95% level; SL = sublingual; i.v. = intravenous; SVRI = systemic vascular resistance index in dynes/sec per m²; WHVP = wedged hepatic venous pressure in mmHg; HVPG = wedged minus free hepatic venous pressure in mmHg; AzBF = azygos blood flow in ml/min; ELBF = estimated liver blood flow in ml/min (ICG method); HR = heart rate in beats/min; MABP = mean arterial blood pressure in mmHg; CO(a)/CI(b) = cardiac output(a)/cardiac index(b) in l/min; IOVP = intraoesophageal variceal pressure in mmHg

to portal vein ligated rats were shown to reduce resistance across collateral vessels (Blei and Gottstein, 1986b).

Acute effects of nitrates on portal and systemic haemodynamics - human studies

The techniques available to estimate hepatic venous pressure gradient, azygos vein blood flow and intraoesophageal variceal pressure are described above.

Five studies have examined the systemic and portal haemodynamic effects of nitroglycerin in patients with cirrhosis (Table 4) and three showed a reduction of HVPG following nitroglycerin administration (Garcia-Tsao et al, 1987; Westaby et al, 1988; Iwao et al, 1991). The effect of nitroglycerin on azygos blood flow was highly variable including some individuals in whom flow increased suggesting vasodilatation of portasystemic collaterals. This suggested that, as observed with experimental animals, the portal hypotensive effect of nitroglycerin could be due to more than one mechanism. Putative mechanisms include:

- 1) Vasorelaxation at a collateral and sinusoidal level leading to reduced resistance to flow through the portal-collateral circulation and also reduced portal pressure.
- 2) Relaxation of arterial smooth muscle which lowers the arterial blood pressure, therefore triggering high pressure arterial baroreceptors to cause reflex splanchnic vasoconstriction. This would reduce portal inflow and hence portal pressure.

- 3) Systemic venodilation reduces the cardiac preload and therefore activates low pressure cardiopulmonary baroreceptors. This would also cause reflex splanchnic vasoconstriction, reducing the portal venous inflow and thus reducing portal pressure.

The effect of different doses of nitroglycerin has been found to be heterogeneous (Rector et al, 1990) suggesting that different doses might act by different mechanisms.

Seven groups have examined the acute haemodynamic effects on cirrhotic patients given isosorbide dinitrate by a variety of routes (Table 5a & b). Only one group (Dawson et al, 1985) showed no effect on HVPG; all others showed a reduction in HVPG which was accompanied by a fall in cardiac index and mean arterial blood pressure. The reduction of HVPG was achieved by a significant fall in WHVP rather than a rise in FHVP.

The amplitude of the haemodynamic response in some patients to isosorbide dinitrate might vary according to the size of portasystemic shunt; bioavailability of the dinitrate is increased as the portasystemic shunt increases (Blei et al, 1984).

Table 6 shows that Is-5-Mn has the same portal and systemic haemodynamic effect as the dinitrate, with the exception of one study (Tsai et al, 1989) of Hepatitis B Antigen positive patients with cirrhosis. Unlike other studies these Hepatitis B positive patients were predominantly Childs Grade A, given premedication and WHVP was assessed by a percutaneous method with a high

Table 5a

THE ACUTE PORTAL AND SYSTEMIC HAEMODYNAMIC EFFECT OF ISOSORBIDE DINITRATE ADMINISTRATION TO PATIENTS WITH CIRRHOSIS

	Halleman, 1983	Dawson, 1985	Freeman, 1985	Merkel, 1987
Number of patients	10	8	10	13
Dose, route	5mg, SL	5mg, oral	10mg, i.v.	5mg, SL
Time	10 mins	30 mins	30 mins	20-30 mins
SVRI	1964-1803			2080-1848*
WHVP			19.4-12.5*	24.8-15.6*
HVPG	17.3 - 13.6*	22-23	14.7-8.6*	20.3-13.5*
AzBF				
ELBF				1384-1284
HR	86-88			83-87*
MABP	81-65*	123-88 (systolic)		100-73*
CO(a) / CI(b)	3.7-3.2* (b)			3.92-3.26* (b)

KEY: * denotes significant difference at least at 95% level; SL = sublingual; i.v. = intravenous; SVRI = systemic vascular resistance index in dynes/sec per m²; WHVP = wedged hepatic venous pressure in mmHg; HVPG = wedged minus free hepatic venous pressure in mmHg; AzBF = azygos blood flow in ml/min; ELBF = estimated liver blood flow in ml/min (ICG method); HR = heart rate in beats/min; MABP = mean arterial blood pressure in mmHg; CO/CI = cardiac output/cardiac index in l/min

Table 5b

THE ACUTE PORTAL AND SYSTEMIC HAEMODYNAMIC EFFECT OF ISOSORBIDE DINITRATE ADMINISTRATION TO PATIENTS WITH CIRRHOSIS

	Blei, 1987			Qureshi, 1988	Mols, 1989	Bhatia, 1990	Merkel, 1990
Number of patients	7		8	6	17	13	
Dose, route	10 mg, oral	40mg, oral		5mg, SL	5mg, SL	5mg, SL	5mg, SL
Time	1 hr	4 hrs	1 hr	20 mins	15 mins	20 mins	25-35 mins
SVRI				24.6-18.5*	1654-1537		
WHVP	22.2-20.5*	22.2-21.4	23.7-20.1*		28-24*		
HVPG	15.4-12.3*	15.4-13.1	16.6-13.7*		18-14*		
AzBF							No change in plasma clearance of ICG
ELBF					650-710		
HR	70-72	70-72	82-86		86-94*	92.5-102.6*	
MABP	90-76*	90-86*	89-72*		89-74*	85.8-80.4*	
CO(a) / CI(b)	4.71-3.63* (b)	4.71-3.81* (b)	4.92-4.02* (b)		4.2-3.7 (b)		
Intrasplenic pulp pressure						43.6 - 35.6*	

KEY: * denotes significant difference at least at 95% level; SL = sublingual; i.v. = intravenous; SVRI = systemic vascular resistance index in dynes/sec per m²; WHVP = wedged hepatic venous pressure in mmHg; HVPG = wedged minus free hepatic venous pressure in mmHg; AzBF = azygos blood flow in ml/min; ELBF = estimated liver blood flow in ml/min (ICG method); HR = heart rate in beats/min; MABP = mean arterial blood pressure in mmHg; CO/CI = cardiac output/cardiac index in l/min

procedural failure rate (Tsai et al, 1989). As Hepatitis B positive cirrhosis and alcoholic cirrhosis have such similar haemodynamic abnormalities it would seem unlikely that the difference in results can be explained simply by the different aetiologies of cirrhosis.

An illustration of different dose dependent mechanisms of the HVPg reducing action of Is-5-Mn may be seen by the work of Navasa et al (1989). The changes in cardiac output and systemic resistance were similar with either 20 or 40 mg of Is-5-Mn but after 40 mg Is-5-Mn there were significantly greater reductions in HVPg, arterial blood pressure and azygos blood flow.

These results are divergent from the study of Hayes and colleagues (1988) which indicated that following administration of 20 mg Is-5-Mn the fall in HVPg was associated with a fall in liver blood flow secondary to baroreceptor mediated splanchnic vasoconstriction, a response only observed with 40 mg of nitrate in Navasa's study. However differences between studies may be related to different severities of liver disease (and different volumes of distribution of nitrate), different degrees of portasystemic shunting, timing of Is-5-Mn administration or different variceal bleeding histories.

To confuse the analysis of mechanism of response to nitrates further, cardiovascular responsiveness to reflex autonomic stimulation is significantly impaired in patients with cirrhosis compared with control subjects (Moreau et al, 1989) and response to nitrates may also vary according to baseline cardiac

filling pressure (Rector et al, 1990) or Child-Pugh classification (Braillon et al, 1986).

Despite the potential complexity of action of nitrates in portal hypertension the overall results of studies of acute administration of nitrates have been remarkably uniform (Tables 5a & b and 6).

It is important to note that the fall in HVPG seen after nitrates compares favourably with the effect of propranolol but that following nitrate administration the fall in HVPG was achieved by a fall in WHVP alone, unlike propranolol which reduces HVPG by both a fall in WHVP and a rise in FHVP (Bosch et al, 1984a; Garcia-Tsao et al 1986; Groszmann, 1984). Reducing WHVP may be more important than reducing HVPG in protection against variceal bleeds (Bosch, 1985; Bosch et al, 1988a).

Effect of organic nitrates on liver blood flow and hepatic resistance

Calculations of hepatic resistance from data in Tables 4 to 8 show that administration of organic nitrates either acutely or chronically induces a significant fall in portal pressure that is largely due to a reduction in resistance rather than to a reduction in liver blood flow, which would be undesirable.

This implies either that portal flow is not reduced or that a compensatory increase in hepatic arterial flow takes place i.e. reciprocity. The difficulty in distinguishing between these two inputs has long been a problem in studies of splanchnic haemodynamics and limits our ability to interpret this data further.

Table 6 THE ACUTE PORTAL AND SYSTEMIC HAEMODYNAMIC EFFECT OF ISOSORBIDE-5-MONONITRATE ADMINISTRATION TO PATIENTS WITH CIRRHOSIS

	Hayes, 1988	Tsai, 1989	Navasa, 1989	
No. of patients	11	10	23	
Dose, route	20mg, Is-5-Mn	20mg, Is-5-Mn	20mg, Is-5-Mn or	40mg, Is-5-Mn
Time	60 mins	60 mins	60 mins	60 mins
SVRI	1831-2030*	1131-1040	899-994*	785-746
WHVP	33.3-29.8*	21.8-21.0	23.5-21.9*	26.9-23.3*
HVPG	23.9-21.8*	17.8-16.3	18.3-16.5*	19.9-16.1*
AzBF			0.52-0.51	0.74-0.62*
ELBF	1940-1639*		890-1003*	1320-1230
HR	85-89.7*	71-72	90.4-90.7	85.8-84.8
MABP	90-88.9	92-82*	88.4-81.3*	87.8-69.8*
CO(a) / CI(b)	3.89-3.42* (b)	4.1-3.9 (b)	8.0-6.8* (a)	8.9-7.7* (a)

KEY: * denotes significant difference at least at 95% level; SVRI = systemic vascular resistance index in dynes/sec per m²; WHVP = wedged hepatic venous pressure in mmHg; HVPG = wedged minus free hepatic venous pressure in mmHg; AzBF = azygos blood flow in ml/min; ELBF = estimated liver blood flow in ml/min (ICG method); HR = heart rate in beats/min; MABP = mean arterial blood pressure in mmHg; CO/CI = cardiac output/cardiac index in l/min; Is-5-Mn = isosorbide-5-mononitrate

Effect of organic nitrates on azygos (collateral) blood flow

The rationale for the pharmacological treatment of oesophageal varices was originally based on the observation that drugs could reduce HVPG (Lebrec et al, 1982). Propranolol, which is discussed above, reduces the HVPG in only 60% of patients with cirrhosis but reduces variceal flow, as measured by azygos flow, in all (Lebrec et al, 1982). It remains unclear whether the major benefit of propranolol, which reduces primary and secondary variceal bleeding (Hayes et al, 1990), is by HVPG reduction or reduced azygos blood flow. Since the HVPG reduction is small and variable, the effect on azygos flow may well be more important.

Navasa et al (1989), showed that azygos blood flow remained unchanged after administration of 20 mg Is-5-Mn despite a significant reduction in HVPG and cardiac output. As the efficacy of vasoactive drugs in either controlling an acute variceal bleed or preventing recurrent haemorrhage may be related to their ability to reduce blood flow through collateral vessels, determination of the effects of vasoactive agents on both collateral blood flow and HVPG is important in evaluating their therapeutic potential. In this respect the change in azygos flow to nitrates is seen to be highly variable and probably dose-dependent (Tables 5-8) and it would seem important to find criteria which would make possible the selection of a dose of nitrate for an individual that both reduces HVPG and azygos blood flow in order to properly assess its efficacy in reduction of variceal bleeding in future clinical trials. Clinically it would be advantageous if an end-point for therapy could be determined

Table 7

THE CHRONIC PORTAL AND SYSTEMIC HAEMODYNAMIC EFFECT OF ORAL ISOSORBIDE DINITRATE ADMINISTRATION TO PATIENTS WITH CIRRHOSIS

	Freeman, 1985	Cervinka, 1989	Ikegami, 1992	Vorobioff, 1992
No. of patients	6	10	9	10
Dose, route	20mg qds	80mg/day, slow release form	40mg/day	15mg/day increasing every 3-4 days to a max of 82±10mg/day
Time	30 days, measured 3-4 hrs after am dose of drug	14 days	4 weeks	65±23 days
SVRI			1308-1178	
WHVP	18.1-9.8*	32.9-17.9*	23.7-21.3*	25.7-22.5*
HVPG	12.6-5.4*	20-9.0	15.6-12.8*	18.9-16.5
AzBF				
ELBF			720-710	
HR		"increased 3-5 bpm"	73-71	87-92
MABP		80.3-76.8	97-87.0*	95-90
CO(a) / CI(b)			5.7-5.7 (a)	

KEY: * denotes significant difference at least at 95% level; SVRI = systemic vascular resistance index in dynes/sec per m²; WHVP = wedged hepatic venous pressure in mmHg; HVPG = wedged minus free hepatic venous pressure in mmHg; AzBF = azygos blood flow in ml/min; ELBF = estimated liver blood flow in ml/min (ICG method); HR = heart rate in beats/min; MABP = mean arterial blood pressure in mmHg; CO/CI = cardiac output/cardiac index in l/min

Table 8

**THE CHRONIC PORTAL AND SYSTEMIC
HAEMODYNAMIC EFFECT OF ORAL
ISOSORBIDE-5-MONONITRATE
ADMINISTRATION TO PATIENTS WITH
CIRRHOSIS**

	Tsai, 1989	Garcia-Pagan, 1990a
No of patients	6	11
Dose, route	20mg Is-5-Mn bd	40mg Is-5-Mn bd
Time	1 week	3 months
SVRI	1131-1177	950-915
WHVP	21.8-18.5	25.5-24.9
HVPG	17.8-15.2	18.6-17.2*
AzBF		0.58-0.52
ELBF		1320-1510*
HR	71-68	77-83*
MABP	92-91	89.4-82.6*
CO(a) / CI(b)	4.1-3.6 (b)	7.5-7.2 (a)

KEY: * denotes significant difference at least at 95% level; SVRI = systemic vascular resistance index in dynes/sec per m⁵; WHVP = wedged hepatic venous pressure in mmHg; HVPG = wedged minus free hepatic venous pressure in mmHg; AzBF = azygos blood flow in ml/min; ELBF = estimated liver blood flow in ml/min (ICG method); HR = heart rate in beats/min; MABP = mean arterial blood pressure in mmHg; CO/CI = cardiac output/cardiac index in l/min; Is-5-Mn = isosorbide-5-mononitrate

non-invasively for example by an increase in heart rate or reduction of mean arterial blood pressure.

Chronic effect of organic nitrates - is tolerance a problem ?

Tachyphylaxis to nitrate preparations is well recognised in patients with heart disease given nitrates for 18 hours or longer (Elkayam et al, 1987; Jugdutt and Warnica, 1989). Organic nitrates are metabolised by vascular smooth muscle as well as by the liver (Fung, 1984) and tolerance has been shown to be associated with an alteration of the vascular metabolism of the nitrate administered (Fung, 1984; Abrams, 1986); systemic clearance of administered nitrate diminishes and the systemic arteriovenous nitrate gradient narrows i.e. with long term dosing plasma nitrate concentrations rise over time. The cyclic-GMP response to nitrates is also diminished in the presence of tolerance.

Administration of exogenous thiols e.g. N-acetylcysteine enhances the nitrate vascular effects in the non-tolerant state (Packer et al, 1986) and removal of organic nitrates from contact with the blood vessel wall for a period of time restores vascular responsiveness.

Studies with isolated rat abdominal aortic rings (with endothelium) showed they retained the repair mechanism to overcome tolerance without the need for exogenous factors which suggests regulation of vascular responsiveness is at the vascular level. Nitrate tolerance appears to be related to reduced sulphydryl availability within vascular tissue and SH groups are required for vasodilatation.

It is important that the effect of nitrate on portal pressure and collateral blood flow after chronic administration is assessed as tolerance would be of concern in the use of nitrates for prophylaxis of variceal bleeding . Experience (Tables 7 and 8) to date shows that a sustained fall in HVPG is seen after chronic nitrate administration in cirrhotic patients which suggests that full tolerance is not of concern in such patients, although partial tolerance has been reported (Garcia-Pagan et al, 1990a).

Therapeutic potential of organic nitrates in liver disease

1) Nitrates in the management of acute variceal bleeding

The most effective treatment for an acute variceal bleed is either injection sclerotherapy (Westaby et al, 1989) or banding (Steigman et al, 1992; Gimson et al, 1993) but they are only effective if promptly applied by experienced personnel. Balloon tamponade has been shown to be as effective as sclerotherapy (Hunt et al, 1982) but is similarly only effective in proficient hands, many patients tolerate the balloon poorly and rebleeding within hours of balloon removal is common (Panes et al, 1988).

It would therefore be desirable if drug therapy was easy to use and efficacious in controlling bleeding, particularly for centres with limited experience of treating variceal bleeds. Acute bleeding, where the above measures are not available, has traditionally been controlled by vasoconstrictor therapy e.g. vasopressin but it is not always effective and has deleterious side-effects (Grace, 1980; Bosch et al, 1981; Kravetz et al, 1984). Several studies have shown that the addition of

nitrates to vasopressin maintains splanchnic vasoconstriction while counteracting some of the deleterious systemic haemodynamic effects such as reduced cardiac output and hypertension, and provides an additional reduction in HVPg (Tsai et al, 1986; Gimson et al, 1986; Westaby et al, 1988; Iwao et al, 1992).

The side-effect profile of vasopressin has prompted pharmacological manipulation in an attempt to maintain efficacy but reduce complications. Glypressin is an analogue of vasopressin (Kyncl et al, 1974) which may have less side-effects than vasopressin (Kravetz et al, 1988) but no study comparing them with vasopressin plus nitrates has yet been reported.

Currently, the combination of vasopressin and a nitrate for treatment of an acute variceal bleed where experienced help is not immediately available merits consideration. Transdermal nitrate patches have been advocated but much more reliable plasma nitrate levels are obtained by the intravenous route (Fung et al, 1983 and 1984) especially in a shocked patient with compromised skin blood flow (Blei et al, 1988). In the acute setting vasopressin should be used at a dose of 0.4-0.8 U/min over 12 hours plus a nitroglycerin infusion at 0.2 mg/min for the duration of the vasopressin infusion.

2) Nitrates for the prevention of rebleeding or prophylaxis of first haemorrhage
Propranolol reduces the frequency of variceal haemorrhage (Lebrec et al, 1981) but has less influence on mortality rates than might be expected (Hayes et al, 1990). Experience of use of nitrates in these two clinical situations is still

limited: there is one trial comparing Is-5-Mn with propranolol suggesting that the two drugs were of similar efficacy in preventing variceal bleeding but further studies will be required (Angelico et al, 1993).

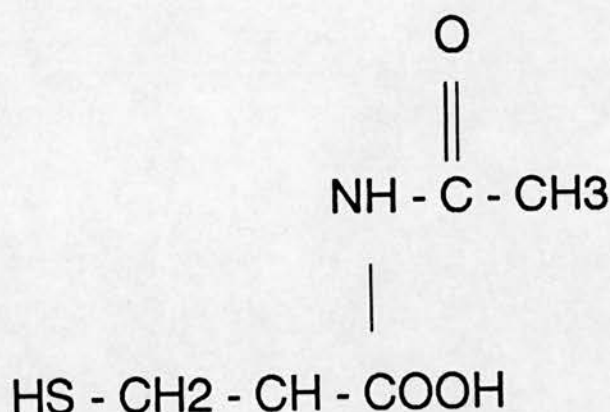
Most literature to date has focussed on the haemodynamic mechanism of action of nitrates and this suggests that rather than a fixed dose, nitrates may need to be individually titrated to obtain maximum benefits without adverse systemic haemodynamic effects. The long term effects of reducing arterial blood pressure in cirrhotic patients is unknown.

Because nitrates reduce HVPg in virtually all patients with cirrhosis they may hold significant advantages over beta-blockade. However, their use as a first line agent in prophylaxis of bleeding cannot be advocated until randomised, controlled clinical trials are performed and the appropriate dose to use is determined.

THE PHARMACOLOGY OF N-ACETYLCYSTEINE AND ITS CLINICAL USES

N-ACETYL-L-CYSTEINE (NAC)

This substance has a molecular weight of 163.2 D and a structure:



It is a synthetic derivative of the naturally occurring amino acid L-cysteine.

Few studies of the plasma concentrations and pharmacokinetics of N-acetylcysteine in man are available. This has largely been as a result of methodological difficulties in assay of NAC. Earlier studies in animals and man used radiolabelled NAC and thin layer chromatography for the separation and identification of NAC and its metabolites (Scheffner et al, 1966; Rodenstein et al, 1978; Bonanomi and Gazzaniga, 1980): cysteine and cystine were identified as the major metabolites of NAC. Inorganic sulphate was the major urinary product excreted together with small amounts of taurine and unchanged NAC (Scheffner et al, 1966).

In animals, N-acetylcysteine is rapidly deacetylated to cysteine and oxidised to disulphides (Johnston et al, 1983).

N-acetylcysteine may be assayed in plasma and urine by gas liquid (Morgan et al, 1983) and high performance liquid chromatography (Lewis et al, 1984) with detection limits of 50 $\mu\text{g/L}$. In plasma NAC can be present in its intact, reduced form as well as in various oxidised forms (Olsson et al, 1988). N-acetylcysteine appears to be extensively bound to plasma (~ 78%) and to tissue proteins by labile disulphide bonds. This is not the same kind of protein binding as exhibited by other drugs (Olsson et al, 1988).

Considerably higher concentrations can be measured by the use of affinity phase chromatography and other thiols to displace drug bound to plasma proteins (Morgan et al, 1983). Its volume of distribution in healthy volunteers is approximately 500 ml/Kg.

The drug disappears rapidly from plasma with a half-life of 0.5 to 6.6 (mean 2-6) hours in healthy volunteers. Previous studies show for healthy volunteers given intravenous N-acetylcysteine that pharmacokinetics fit either bi or tri-exponential models (Borgstroem et al, 1986; Olsson et al, 1988).

No data on the pharmacokinetics of N-acetylcysteine in chronic liver disease has been published prior to our study reported here. The elimination of

N-acetylcysteine has not been found to be impaired in patients with paracetamol poisoning treated late and who suffered severe liver damage (Prescott et al, 1989).

Many drugs however have altered pharmacokinetics in the presence of chronic liver disease, for example, d-propranolol (Passayre et al, 1978) and bisoprolol (Hayes et al, 1987).

For intravenous use in the treatment of paracetamol poisoning, there is a pyrogen free solution containing 200 mg N-acetylcysteine per ml in ampoules of 10 ml: Parvolex (Duncan, Flockart, UK). This is the formulation used in our studies.

Use of N-acetylcysteine in cardiology patients

Evidence has shown that N-acetylcysteine is capable of reversing the nitrate tolerance seen in patients with stable and unstable angina (Horowitz et al, 1988a; Boesgaard et al, 1992), although this is controversial (Hogan et al, 1990). The molecular basis for reversal of tolerance appears to be sulphhydryl donation by N-acetylcysteine (Fung et al, 1988). N-acetylcysteine has also been shown to potentiate the coronary vasodilative effect of nitroglycerin (Winniford et al, 1986).

Recent evidence suggests that combined therapy with nitroglycerin and intravenous N-acetylcysteine results in potentiation of haemodynamic responses to nitroglycerin and reduces the incidence of myocardial infarction in patients with severe unstable angina pectoris (Horowitz et al, 1988a and b and 1991).

This is in contrast to patients undergoing cardiac catheterisation for evaluation of chest pain where administration of N-acetylcysteine alone intravenously had "no consistent haemodynamic effect" (Horowitz et al, 1983) and in eight patients with

severe chronic heart failure where N-acetylcysteine alone "had little haemodynamic effect in patients not receiving nitroglycerin" (Packer et al, 1987).

In one study N-acetylcysteine appeared not to reverse tolerance to intravenous nitroglycerin in patients with congestive heart failure (Dupuis et al, 1990) but another study has shown partial reversal of tolerance (Packer et al, 1987).

There is some evidence that susceptibility to the development of nitrate tolerance in humans is higher in the venous than in the arterial circulation, and that N-acetylcysteine is extremely effective in reversing nitroglycerin tolerance in the venous circulation in humans (Ghio et al, 1992).

N-acetylcysteine in the healthy state

It is controversial whether N-acetylcysteine possesses direct relaxant activity in normal vascular smooth muscle: Horowitz et al (1983) and Torresi et al (1985) suggest that it has no direct relaxant activity but a recent study (Sunman et al, 1993) showed N-acetylcysteine caused an endothelium independent relaxation of rat and human arteries. Three control pigs demonstrated no physiological change after NAC infusion in a different study (Modig and Sandin, 1988).

Administration of NAC had no significant systemic haemodynamic effect in 4 patients who had fully recovered from hepatic failure at Kings College Hospital (Harrison et al, 1991).

Hogan et al (1989) demonstrated tolerance to the haemodynamic effects of GTN patches in normal volunteers with chronic dosing but concomitant dosing with 200 mg NAC failed to alter this phenomenon. The literature to date therefore, though not extensive, suggests that NAC has no haemodynamic effect in healthy controls.

Use of N-acetylcysteine in patients with fulminant hepatic failure

Keays et al (1991), in a prospective controlled trial reported that N-acetylcysteine treated patients with fulminant hepatic failure showed increased survival, a lower incidence of cerebral oedema and less requirement for inotropic support than controls not given NAC. However there are a number of criticisms of this study which make interpretation of all data difficult - there were too many variables (blood/albumin infusion, haemodialysis/haemoperfusion, ventilation/not), each patient had treatment beginning at a variable time point from the moment of ingestion of paracetamol and this interval and the length of treatment with NAC was not stated in the paper. Requirement for inotropes and renal support may be less in the NAC treated group than controls because NAC is a positive inotrope rather than by treatment of underlying disease processes (Harrison et al, 1991).

N-acetylcysteine has been reported to increase cardiac output and tissue oxygen delivery and utilisation in fulminant hepatic failure (Harrison et al, 1991), mostly due to paracetamol poisoning. It also resulted in reduction of the pulmonary and systemic vascular resistance index in such patients (Harrison et al, 1991).

Interestingly, there were no reported changes in arterial pO_2 , calculated arterio-venous tension gradient and shunt in hepatic failure patients in response to NAC.

Use of N-acetylcysteine in paracetamol poisoning

N-acetylcysteine is used primarily for the treatment of paracetamol overdosage where plasma paracetamol concentrations are above a line on a semi-logarithmic graph joining plots of 200 $\mu\text{g/ml}$ at 4 hours and 30 $\mu\text{g/ml}$ at 11 hours after ingestion of the paracetamol. Early treatment (8-10 hours post overdose) with N-acetylcysteine prevents hepatic necrosis, acute renal failure and death following paracetamol overdosage; use after this time is more controversial. An initial loading dose of 50 mg/Kg is given over 15 minutes followed by infusion of 50 mg/Kg over 4 hours and 100 mg/Kg over the next 16 hours.

At the dose used for the treatment of paracetamol poisoning NAC has no hepatotoxic effects, in terms of glutathione-S-transferase rise, a sensitive marker of hepatocellular integrity (Beckett et al, 1990). It is readily converted to cysteine in vivo and by stimulating hepatic glutathione synthesis it effectively prevents acute paracetamol-induced hepatotoxicity (Burgunder et al, 1989). Glutathione plays a vital protective role by preferential conjugation with the toxic alkylating metabolite of paracetamol and liver damage does not occur unless it is depleted (Mitchell et al, 1974).

Other uses

By virtue of its free thiol group, N-acetylcysteine can form stable mercaptides with heavy metals and has had limited use in the treatment of poisoning with these agents (Flanagan, 1987).

Oral N-acetylcysteine reduces sputum viscosity and facilitates expectoration in patients with bronchopulmonary disease with improvement in symptoms and lung function tests. It has proven efficacy in combination with hypermellose as eye drops in keratoconjunctivitis sicca (Absolon and Brown, 1968).

Uncontrolled studies suggest that oral and rectal N-acetylcysteine can relieve the meconium ileus equivalent in patients with cystic fibrosis (Hodson et al, 1976).

N-acetylcysteine protects against the acrolein-induced haemorrhagic cystitis caused by cyclophosphamide and isophosphamide without reducing anti-tumour activity (Slavik and Saiers, 1983).

Recent evidence suggests that N-acetylcysteine is a potent suppressor of human immunodeficiency virus transcription in persistently infected cells (Kinter et al, 1992). The clinical significance of this observation remains to be established.

Adverse reactions to N-acetylcysteine

There have been occasional reports of "anaphylactoid" reactions to intravenous N-acetylcysteine in patients with paracetamol overdose, characterised by rash, flushing and less commonly, angioedema, bronchospasm, and hypotension (Mant et al, 1984; Ho and Beilin, 1983). They are usually readily reversible and mild. Flushing is a common effect seen in patients given intravenous N-acetylcysteine for paracetamol poisoning.

THE PHARMACOKINETICS OF DRUGS IN CHRONIC LIVER DISEASE

The pharmacokinetics of organic nitrates

The pharmacology of isosorbide dinitrate and Is-5-Mn nitrates is well described in the literature.

Is-5-Mn has a prolonged half-life (5h) (Abshagen et al, 1981; Taylor et al, 1981; Abshagen et al, 1992) and dose-linear kinetics (Abshagen, 1986) even in the presence of liver disease (Steudel et al, 1983; Akpan et al, 1985).

In contrast, the dinitrate has a high first-pass effect with an oral bioavailability of 0.2 in normal subjects (Fung, 1985) which increases up to a mean of 0.65 in cirrhosis because of reduced hepatic extraction and the presence of porta-systemic shunts (Blei et al, 1987) in which there is interindividual variation. As the pharmacokinetics of nitrates in the presence of chronic liver disease were so well described in the literature, we did not undertake further study of this.

The pharmacokinetics of N-acetylcysteine

However the opposite is true for N-acetylcysteine where due to methodological difficulties with assay of the drug, few studies on pharmacokinetics in man have been reported and none in the presence of chronic liver disease.

The method of analysis is very important in NAC estimations. For example Borgstroem et al (1986 and 1990) assayed NAC in deproteinised plasma. As NAC is bound to thiols and proteins this method does not measure TOTAL plasma NAC.

However the method of Lewis et al (1984) and the modification of that method (Prescott et al, 1989) measured TOTAL plasma NAC, ie total oxidised and reduced NAC and NAC bound to other thiols and proteins, and therefore it is difficult to make comparisons between different pharmacokinetic studies.

Borgstroem et al (1986) found the kinetics in normal controls conformed to a triphasic pattern but Prescott et al found a biphasic pattern (2 compartment model) in patients with paracetamol poisoning. The estimated half-life of the drug is broadly comparable between the two studies. The volume of distribution of the drug in normal controls and paracetamol poisoning was consistent with a distribution mainly to extracellular water.

The metabolism of N-acetylcysteine

Radiolabelled NAC studies and other studies have shown that 20-30% of an intravenous dose is excreted unchanged in the urine i.e. 70% is cleared by extra-renal routes (Borgstroem et al, 1986).

NAC is deacetylated in the liver in the rat, mouse and human tissue in vitro (Sjodin et al, 1989) but it is also rapidly metabolised to cysteine and inorganic sulphate in the gastrointestinal tract (Cotgreave et al, 1987). It has been shown in rats that only a small amount (3%) of radioactive NAC is excreted in faeces, even after intravenous and oral administration (Bonanomi and Gazzaniga, 1980); the low availability after oral administration is probably due to fast metabolism in the gut wall and liver. This could be studied by measuring the plasma concentration of NAC after oral administration in TIPSS patients.

Once absorbed, N-acetylcysteine appears to be deacetylated to cysteine (Scheffner et al, 1966) and used in the synthesis of glutathione or other sulphurated compounds (Lauterburg et al, 1983). This metabolic step is probably a prerequisite for most of the protective effect of NAC in paracetamol poisoning, since these effects are thought to be dependent on de novo glutathione biosynthesis (Lauterburg et al, 1983; Corcoran and Wong, 1986).

The deacetylation appears to be a process with rather specific structural requirements on the substrate, since the D isomer of NAC and the disulphide of L-NAC proved resistant to deacetylation (hydrolysis). Other N-acetylated amino acids have been shown to be stereoselectively hydrolysed (Birnbaum, 1952). The enzyme responsible for the deacetylation (hydrolysis) of NAC seems uncertain. Coenzyme A dependent N-acetyltransferase and microsomal deacetylases are known to acetylate several xenobiotics (Weber and Hein, 1985) but have not been shown to catalyse deacetylation; they would therefore seem unlikely candidates.

A large number of proteins in animal and plant cells are N-acetylated on their N-terminal amino-acid (Tsunasawa and Sakiyama, 1984). The enzyme, acyl-peptide hydrolase from rat liver, liberating the N-acetylated amino-acid has been reported (Tsunasawa et al, 1975; Kobayashi and Smith, 1987). This appears to have a physiological role in the regeneration of free L-amino acids during protein turnover and may well be responsible for the hydrolysis of NAC (Sjodin et al, 1989).

In vivo, organs other than the intestine most likely contribute to the biotransformation of NAC but the gut or liver clearly play a significant role since after intravenous infusion of 600 mg NAC into human volunteers, 30% of unchanged drug or its disulphide form was found in the urine but after oral intake around 3% was found in the urine i.e. high first pass clearance (Borgstroem et al, 1986). Deacetylation may be the initial metabolic step, but in vivo other reactions like S-methylation and S-oxidation may take place as well.

The liver metabolism of drugs and mechanisms of altered pharmacokinetics in chronic liver disease

Many factors determine the elimination of a drug from the body, including its rate of absorption, its distribution in body fluids, its binding to plasma proteins and its metabolism and elimination by particular organs such as the liver and kidneys. The liver is particularly important in the process as it metabolises most drugs to some extent.

The extraction ratio of a drug across an eliminating organ can be calculated by clearance flow through an organ. Drugs may be classified in relation to their clearance from the blood as highly cleared ($> 70\%$ of drug cleared at each passage through the liver) or poorly cleared ($< 30\%$), or intermediate. Examples of drugs with high and low hepatic clearances are shown in Table 9.

Hepatic clearance has an important effect on the extent to which drugs become available in the systemic circulation particularly when drugs are given orally;

Table 9

Examples of commonly used drugs with high and low clearances by the liver

High clearance	Low clearance
Chlormethiazole	Ampicillin
Dextropropoxyphene	Chloramphenicol
Domperidone	Chlordiazepoxide
Labetalol	Cimetidine
Lignocaine	Diazepam
Methadone	Digoxin
Midazolam	Frusemide
Morphine	Naproxen
Pethidine	Phenylbutazone
Propranolol	Prenisolone
Verapamil	Spironolactone
	Theophylline
	Tolbutamide
	Warfarin

highly cleared drugs have high "first pass" removal and therefore low systemic availability.

Hepatic clearance of a drug is known to be a function of 2 factors:

- i) liver blood flow
- ii) ability of the liver to remove the drug from the sinusoids

(Wilkinson and Shand, 1976).

In normal liver, the relative importance of each of these factors for a particular drug is dependent on its hepatic extraction ratio. For drugs with high extraction ratios, hepatic blood flow is the major factor determining its elimination. In contrast, the elimination of drugs with low extraction ratios is determined by enzyme activity. The elimination of drugs with intermediate extraction ratios is dependent on both hepatic blood flow and hepatic enzyme activity (Rowland et al, 1973; Nies et al, 1974; Branch et al, 1975) (Figure 7).

However, this concept may be less important in the presence of chronic liver disease as once believed, as Passayre and colleagues (1978) showed in an elegant study that in cirrhosis, d-propranolol (a highly extracted drug in normal controls) is no longer highly extracted and its hepatic clearance depended not only on liver blood flow but also and predominantly on the ability of the liver to remove drug from the blood. This was also reported in an study using isolated perfused normal and cirrhotic rat livers and another "highly extracted drug" meperidine, which is metabolised by oxidation by the liver (Callaghan et al, 1993). Regression analysis showed that in normal livers the elimination of meperidine was mainly dependent

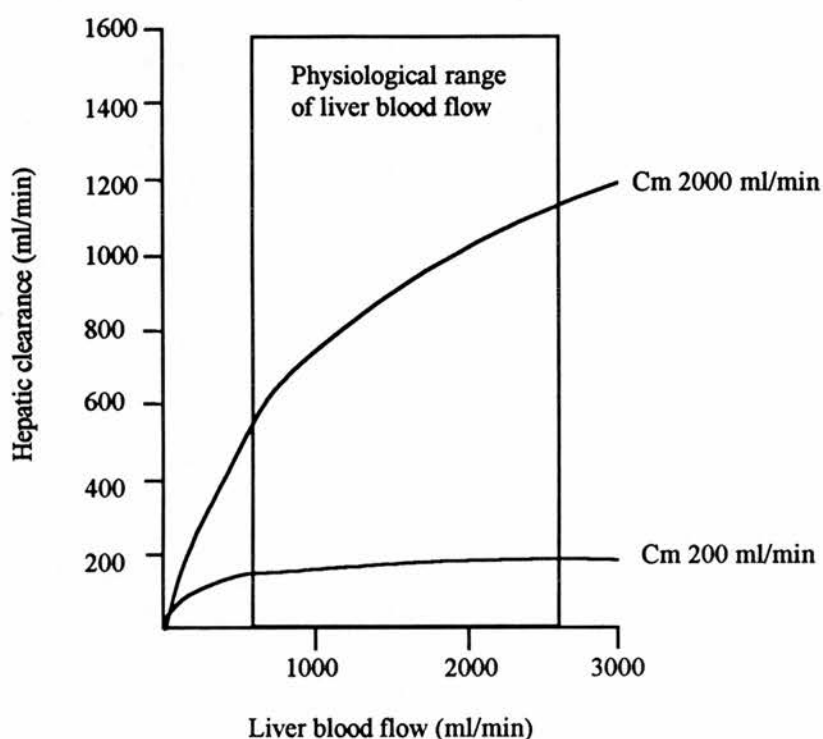


Figure 7

Theoretical effects of altering hepatic blood flow for a drug with a low intrinsic hepatic clearance (200 ml/minute) and a drug with high intrinsic hepatic clearance (2000 ml/minute). C_m = maximum drug clearance (after Branch et al, 1975).

on flow, but in cirrhotic livers the reduced hepatic clearance of meperidine was contributed to by reduction in intrinsic clearance or enzyme activity. The study demonstrated that enzyme activity becomes an important determinant of altered drug disposition in cirrhosis (Callaghan et al, 1993).

Thus the main factor responsible for the reduced clearance of drugs metabolised by the liver in patients with cirrhosis appears to be impaired ability of the liver to remove drug from the blood. This has yet to be explained but possible mechanisms include:

- i) intrahepatic shunts
- ii) portasystemic shunts
- iii) reduced amounts of drug metabolising enzyme molecules
- iv) reduced hepatic uptake of drugs

In discussion of liver metabolism of any drug it must be remembered that the metabolic capacity of the liver is not a homogeneous entity but depends on the metabolic pathways involved. Oxidation (Phase I) takes place in a centrilobular location (Figure 1; Zone 3) and is thus more prone to hypoxia and more affected in liver disease than Conjugation (Phase II) which is periportal in location (Zone 1) and well preserved in liver disease (Callaghan et al, 1993).

Other possible mechanisms which could be responsible for altered kinetics in chronic liver disease include:

i) altered protein binding, affecting the penetration of drugs into tissues and drug distribution may be altered, changing the proportion of drug available for metabolism.

ii) acid-base or electrolyte changes.

The relative importance of alterations in hepatic enzyme activity, blood flow and protein binding to drug elimination in patients with liver disease remains controversial.

Drugs which show reduced clearance in chronic liver disease

A number of other drugs are metabolised by the liver and demonstrate lower clearance in cirrhosis than in control subjects.

These include:

amylobarbitone

antipyrine

bisoprolol (Hayes et al, 1987)

diazepam

glutethimide (Schmid et al, 1964)

* lidocaine

* meperidine (Callaghan et al, 1993)

* d-propranolol (Passayre et al, 1978)

In those marked with a * reduced clearance has been shown by invasive studies to be due to reduced hepatic extraction and blood flow (as discussed above). In the

bisoprolol study (Hayes et al, 1987) it was shown that although clearance was reduced in cirrhosis, gastrointestinal absorption was reduced thereby reducing the likelihood of accumulation of the drug.

An interesting study of the effect of liver disease on plasma levels of amylobarbitone has been reported (Mawer et al, 1972). The serum concentration and the rate constant for the slower phase of the double exponential graph of the logarithm of the concentration in serum against time was reduced in the group of patients with lower albumin concentrations. The slower phase is an indication of the combined rates of metabolism and excretion (the faster phase is an indication of tissue penetration by the drug). The body thus appears to compensate for reduced hepatic function by modification in the rate of drug penetration into tissues and thus the clinical response was the same in patients with liver disease and normal controls.

The effect of liver disease on drug metabolism is very difficult to assess, as explained above, as there are so many confounding variables involved.

Drugs whose pharmacokinetics do not appear to be altered by chronic liver disease

There are a number of drugs for whom no discernible differences have been detected in pharmacokinetics in the presence of chronic liver disease.

These include:

Chloramphenicol (Kunin et al, 1959)

Chlorpromazine (Maxwell et al, 1972)

Digoxin (Marcus and Kapadia, 1964)

Pentobarbitone (Sessions et al, 1954)

Tolbutamide (Nelson, 1964)

In general, these appear to be drugs with low hepatic clearance (Table 9).

ASSESSING EXISTING EVIDENCE

In the review of previous work above, identification of published studies has been made using computerised searches. Basing reviews solely on publications can be misleading, however, because published reports tend to favour studies with "significant" or "promising" results (Simes, 1986). Published trials of treatment for advanced ovarian cancer, for instance, demonstrated significant survival advantage for combination chemotherapy over an initial alkylating agent, whereas a pooled analysis of all trials in a cancer trials registry did not substantiate this conclusion (Simes, 1986). Although identification of unpublished studies may be daunting, for clinical trials at least this task is becoming increasingly feasible with moves to establish prospective registration of all trials.

Identified relevant studies should be therefore critically appraised to assess bias. Standardised forms are helpful, making clear the criteria used (Mulrow, 1987). Although the existing data will often be insufficient to merit meta-analysis, such

pooling should be considered with caution when several good quality studies exist (Thompson and Pocock, 1991).

AIMS OF THE RESEARCH

The aim of my study was to examine the haemodynamic effect of two vasodilator drugs, isosorbide-5-mononitrate and N-acetylcysteine in patients with stable cirrhosis:

- to examine the effect of chronic oral administration of isosorbide-5-mononitrate on portal pressure, liver blood flow and azygos vein blood flow in patients with cirrhosis, with respect to tolerance and dose-dependency (Section II). It was also important to study the effect of rechallenge with the nitrate after chronic therapy.

Extensive work on the pharmacokinetics of isosorbide-5-mononitrate and the effect of nitrates on the systemic circulation, including forearm blood flow has already been reported and therefore was not necessary in my study.

- to examine if N-acetylcysteine, which has been shown to increase cardiac output and tissue oxygen consumption in patients with hepatic failure (Harrison et al, 1991) has the same action in patients with stable cirrhosis and if, as a vasoactive agent, it has any action on the HVPG or liver blood flow (Section III).

Since some of the haemodynamic changes seen in patients with cirrhosis are similar to those with fulminant hepatic failure , for example peripheral vasodilation, our hypothesis is that N-acetylcysteine may have the same action in cirrhosis.

The main determinants of flow through the liver are the hepatic arterial resistance, splanchnic resistance and intrahepatic portal resistance. The regulator with the most physiological variation is splanchnic resistance, which is under neural and hormonal control as it determines the percentage of cardiac output reaching the liver. Therefore studies investigating new drugs need simultaneous determination of cardiac output with the hepatic haemodynamic evaluation. This is even more important in view of the frequent hypercirculatory state in patients with cirrhosis of the liver.

- **to use a forearm blood flow model to examine (without the difficulties of interpreting reflex actions) the direct vascular action of N-acetylcysteine on blood flow of patients with stable cirrhosis and compare this with normal controls (Section IV).**
- **to examine the pharmacokinetics of intravenous N-acetylcysteine administration in patients with cirrhosis compared with that in normal controls (Section V), since it has never previously been described in cirrhosis.**

Section II

**Portal and systemic
haemodynamic response
to acute and chronic
administration of low and
high dose
isosorbide-5-mononitrate
in patients with cirrhosis**

INTRODUCTION

Previous reports show that both isosorbide dinitrate and isosorbide-5-mononitrate are capable of reducing the hepatic venous pressure gradient and/or azygos vein (collateral) blood flow both acutely and with chronic use but the results have been highly variable (Section I). For example, it has been reported that azygos vein blood flow may even increase in some patients in response to nitrates (Navasa et al, 1989; Garcia-Pagan et al, 1990a; Grose et al, 1994).

The main aim of my study was to investigate this variability further; to establish if it was related to:

- a) nitrate dosage, or to
- b) patient factors, such as severity of liver disease.

In addition, no previous studies have examined the chronic effect of Is-5-Mn on the hepatic venous pressure gradient and azygos blood flow using a 16 hour nitrate free dosing interval, particularly with regard to **tolerance**. Rechallenge with the nitrate after omission of the morning dose following 4 weeks of twice per day therapy had also not previously been investigated.

In order to establish whether Is-5-Mn was acting to reduce the hepatic venous pressure gradient (HVPG) by reducing the hepatic vascular resistance, it was considered highly desirable that measurement of liver blood flow by the continuous infusion method (ICG) was made at the same time as HVPG.

MATERIALS AND METHODS

The population studied

25 patients with biopsy-proven cirrhosis (14 men; 11 women) with a range of disease severity (8A; 11B; 6C - Childs'-Pugh Grading) were studied (Appendix Ia and Ib). Exclusion criteria included myocardial infarction, ischaemic heart disease, valvular heart disease, cardiomyopathy, cerebrovascular disease, pregnancy, concurrent vasoactive medication, current viral hepatitis B,C or D or bleeding diathesis (with a prothrombin time ratio greater than 2.5:1).

All patients gave written informed consent and ethical permission was obtained from the Lothian Health Board Medical Ethics Subcommittee. All studies conformed to the Helsinki declaration. Each patient was randomised to receive either 10mg Is-5-Mn or 40mg Is-5-Mn by random number selection from sealed envelopes.

Investigations prior to each study

Routine investigations prior to undertaking each study included estimation of serum urea and electrolytes, a full blood count including haematocrit, "liver function tests" including bilirubin (Bili), alanine aminotransferase (ALT), gamma-glutamyl peptidase (GGT), alkaline phosphatase (Alk.Phos) and albumin (Appendix Ia and Ib). Assessment of the severity of each patients' chronic liver disease was made by Pugh's modification of Child's classification (Section I - Table 3) (Pugh et al, 1973). The low and high dose study groups (10 and 40 mg Is-5-Mn respectively) were not significantly different in terms of age, prothrombin time, aetiology of cirrhosis, Child's-Pugh Score, serum bilirubin

and serum albumin (Appendix II). The sex ratios were different between the two dosage groups; but there has been no evidence for a different haemodynamic response to vasoactive drugs between male and female patients with cirrhosis in the past.

The study procedure

Each patient fasted after a light breakfast on the day of the study. A constant temperature of 25-27°C and as quiet and relaxed an environment as possible was kept in the catheter laboratory. Three lead (Lead II) ECG monitoring was commenced by attachment to a Hewlett Packard HP monitoring system, (Germany) and mean arterial blood pressure (manual syphygmomanometer) was checked every five minutes initially until all patients had achieved haemodynamic stability for at least 20 minutes.

A venflon (21G) was placed in a vein in the antecubital fossa of the right arm and a sample of venous blood was taken as a baseline, against which spectrophotometric measurements were made later. A bolus of 1g Cefotaxime (Roussel Ltd, Uxbridge, Middlesex) in 10ml sterile water was given intravenously as antibacterial prophylaxis to all patients. A loading dose of 0.2 mg/Kg body weight of indocyanine green dye (ICG, Cardiogreen, Hynson, Westcott and Dunning Inc, Baltimore, Md) was also given through the venflon and an infusion of 0.25mg /minute was commenced. 50mg indocyanine green dye was diluted in 20 ml of supplied solvent solution and 20ml 0.9% saline to a final concentration of 1.25 mg/ml. The infusion rate of the ICG solution was 12ml/hour i.e 0.25 mg/minute by accurate infusion pump (Gemini).

A 7.5 F introducer (Edwards, Critical Care Division, Irvine, USA) was then placed in the right femoral vein under local anaesthesia (approx 10 ml of 2% lignocaine). The hepatic venous pressure gradient (wedged hepatic venous pressure minus free hepatic venous pressure) was then estimated by inserting a sidewinder II catheter (Cordis, USA) into the main right hepatic vein under fluoroscopic control. Inflating and releasing the balloon enabled repeated consistent measurements of wedge and free hepatic pressure respectively to be made by connection of the free end of the sidewinder catheter to the Hewlett Packard manometer line (containing 2000 units of heparin in 500 ml 0.9% saline as a "flush"). The Hewlett Packard machine was zeroed for pressure at the level of the right atrium at the start of each procedure. Examples of wedged and free hepatic pressure traces are shown in Appendix III.

After a 20-30 minute equilibration period after commencement of the ICG infusion, three simultaneous samples of peripheral and hepatic venous blood were drawn into syringes for the determination of estimated liver blood flow (constant infusion method; Cherrick et al, 1960; Winkler et al, 1965). These six samples and the "baseline" sample taken earlier were centrifuged at room temperature, for six minutes at 3000 rpm, in a (Beckmann, bench-top centrifuge). 1ml of each serum sample was decanted with a disposable pipette into a cuvette. The ICG concentrations in the serum and the infusion fluid were estimated by absorption spectrophotometry at 805nm in a Zeiss PMQ II spectrophotometer against the blank serum sample. The Estimated Liver Blood

Flow was derived from the equation:

$$ELBF = R/(A-H)(1-Hct)$$

where **R** is the rate of infusion of ICG in mg/min; **A** and **H** are the concentrations of ICG in peripheral and hepatic venous blood respectively in mg/L; and **Hct** is the haematocrit. The optical density vs concentration of ICG graph (data supplied by the manufacturer of ICG) is shown in Appendix IV with a sample calculation of liver blood flow. The infusion of ICG was discontinued after the blood samples had been taken.

The sidewinder II catheter was then removed and an azygos catheter (Webster Lab Inc) was introduced into the azygos vein under fluoroscopic screening. The anatomical position of the azygos vein is shown in Appendix V. The electrical integrity of the thermistor was checked prior to insertion into the patient by a custom built circuit tester made by the Department of Medical Physics.

Estimation of azygos vein blood flow was made by the thermodilution technique (Hayes et al, 1992) using 5% dextrose at room temperature at 50ml/min as injectate. The syringe pump (Harvard model 22 modified to meet BS5724 Standard) was used to deliver the dextrose accurately into the distal lumen of the azygos catheter. The electrical connection of the catheter was connected to a custom-built IBM model PS2-286 microcomputer interface to give real time values for blood flow using the algorithm:

$$F_b = F_i \times C \left\{ \frac{T_i - 1}{T_b} \right\}$$

T_i = change in injectate temperature

T_b = change in blood temperature

C = constant dependent on heating properties of blood and saline and the catheter manufacturer's constant.

(Ganz et al, 1971)

A real-time graphic display of blood flow was recorded for up to one minute until a steady reading was achieved. Data were stored on hard and floppy disks for later analysis. An example of an azygos venous blood flow trace obtained by this method is shown in Appendix VI. The position of the catheter was noted for the next occasion of its use in that patient to minimise any potential effect of changed catheter position on flow.

A baseline heart rate and blood pressure reading was taken prior to the administration of either a 10mg or a 40mg tablet of Is-5-Mn orally, with a small volume of iced water.

Readings of heart rate, blood pressure and azygos vein flow traces were taken exactly thirty minutes and sixty minutes after administration of the drug. Sixty minutes after administration of the drug, the azygos catheter was removed and the Sidewinder catheter was reinserted into the main right hepatic vein and a further estimation of wedged and free hepatic venous pressure and liver blood flow was made by the above methods. The procedure was concluded with the removal of the sidewinder catheter, the femoral introducer and the venflon.

Chronic administration of Is-5-Mn

Each patient was given 100 tablets of their randomised dose of Is-5-Mn (kindly supplied by Boehringer-Mannheim Ltd) and given written instructions to take one tablet in the morning at 9am and the next tablet at 5pm each day i.e. allowing for a nitrate free interval of 16 hours overnight. Each patient was asked to return the box of tablets at the end of the study (for a tablet count). The last morning dose (on the day of the second haemodynamic investigation) was omitted. Patients were warned about potential headaches particularly within the first few days of starting the tablets and were asked to 'phone and report this or any other symptoms. As each patient was very well known to the staff in the unit we did not feel that they would be reticent in reporting side-effects. Each patient was also asked specifically about side-effects on return for the second estimation of portal pressure and azygos blood flow after one month.

The second procedure

The heart rate, blood pressure, hepatic venous pressure gradient, estimated liver blood flow and azygos vein blood flow were remeasured after 28 days of Is-5-Mn therapy both before and after rechallenge with their randomised nitrate dosage.

A summary of the measurements made on each attendance at the catheter laboratory is shown in Table 10.

A SUMMARY OF THE HAEMODYNAMIC MEASUREMENTS MADE ON EACH ATTENDANCE AT THE CATHETER LABORATORY

Table 10

0 mins	30 mins	60 mins	DERIVED DATA
HR	HR	HR	MABP
BP	BP	BP	
AzBF	AzBF	AzBF	
WHVP		WHVP	HVPG
FHVP		FHVP	
ICG conc		ICG conc	
			ELBF

WHERE:

HR= heart rate in bpm

BP = arterial blood pressure in mmHg

MABP = mean arterial blood pressure in mmHg
= diastolic + $\frac{1}{3}$ (systolic - diastolic)

AzBF = azygos blood flow in ml/min

WHVP = wedged hepatic venous pressure in mmHg

FHVP = free hepatic venous pressure in mmHg

HVPG = hepatic venous pressure gradient in mmHg

ICG conc = ICG concentration in mg %

ELBF = estimated liver blood flow in ml/min

Statistical analysis

Results are expressed as mean haemodynamic values for each treatment group \pm standard error of the mean. Each mean variable at one time point was compared with the haemodynamic value at time 0 (i.e. baseline value) by a two-tailed paired students' t-test.

Correlation of change in each haemodynamic variable with serum albumin, bilirubin and prothrombin ratio (PTR) was made using the Spearman Rank Correlation test on "Systat" Version 5.0 for Microsoft Windows on an IBM 486 microcomputer.

RESULTS

Completion of the study

Twelve patients were randomly allocated to receive 10 mg and thirteen 40 mg Is-5-Mn. Four patients (two in each group) failed to attend for the second invasive procedure and in one patient the procedure was abandoned for technical reasons.

Baseline haemodynamic characteristics

No significant difference in baseline haemodynamic characteristics between the two treatment groups was seen (Table 11).

Heart rate response to Is-5-Mn

No significant change in heart rate was seen acutely or after four weeks of 10mg bd Is-5-Mn (Figure 8 and 9). In contrast, patients given 40mg Is-5-Mn showed a significant rise in heart rate 30 and 60 minutes after drug administration (Figure 10) which was still present after a month (28 days) of therapy (Figure 11).

The effect of Is-5-Mn on mean arterial blood pressure

Acute administration of either 10mg or 40 mg Is-5-Mn produced a significant fall in blood pressure after 30 and 60 minutes (Figures 12-15). This fall in blood pressure was not sustained prior to or after rechallenge with the nitrate at 28 days (one month).

The effect of Is-5-Mn on the Hepatic Venous Pressure Gradient (HVPg)

10mg Is-5-Mn had no significant effect on free hepatic venous pressure acutely or after chronic nitrate use (Figure 16). Wedged hepatic venous pressure fell

Table 11 **BASELINE HAEMODYNAMIC CHARACTERISTICS**
OF THE TWO Is-5-Mn STUDY GROUPS

Variable	10 mg Is-5Mn group n = 12	40 mg Is-5-Mn group n = 13	p value
Number in group			
Heart rate (bpm)	78.3 ± 3.5	72.5 ± 3.0	p > 0.1 (NS)
Mean arterial blood pressure (mmHg)	93.7 ± 4.0	96.2 ± 2.2	p > 0.5 (NS)
Wedge hepatic venous pressure (mmHg)	21.4 ± 2.0	23.2 ± 1.6 (n = 12)	p > 0.317 (NS)
Free hepatic venous pressure (mmHg)	5.5 ± 1.2	7.1 ± 1.2	p > 0.317 (NS)
Hepatic venous pressure gradient (mmHg)	15.9 ± 1.8	16.4 ± 0.7 (n = 12)	p > 0.5 (NS)
Azygos vein blood flow (ml/min)	378 ± 91	511 ± 103	p > 0.137 (NS)
Liver blood flow (ml/min)	1227 ± 184 (n = 5)	1313 ± 159 (n = 7)	p > 0.5 (NS)

Heart rate. 10mg Is-5-Mn - first study.

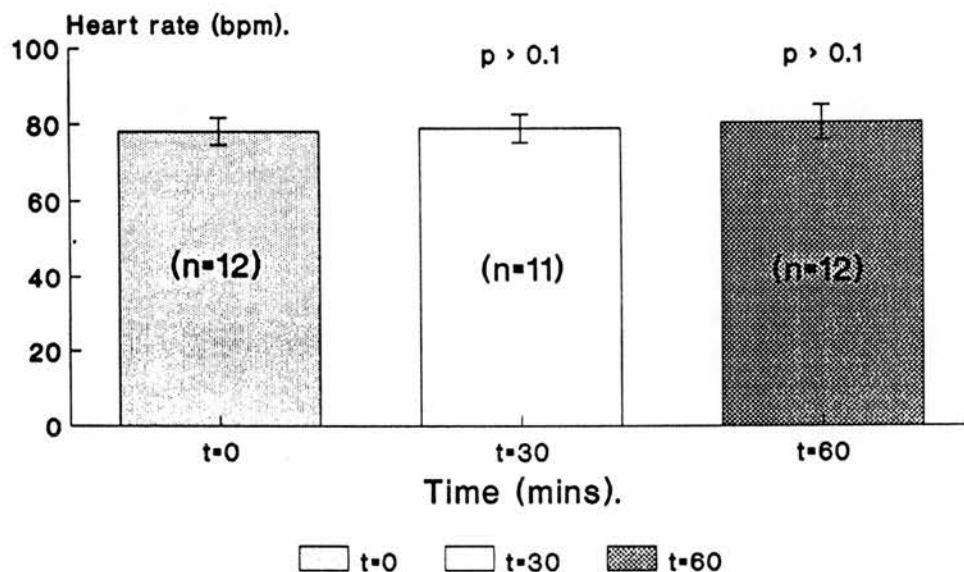


Figure 8

The mean heart rate response to acute administration of 10mg Is-5-Mn in 12 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values at the start of the study.

The number of measurements possible for technical or attendance reasons is included in brackets.

Heart rate.

10mg Is-5-Mn after 1 month.

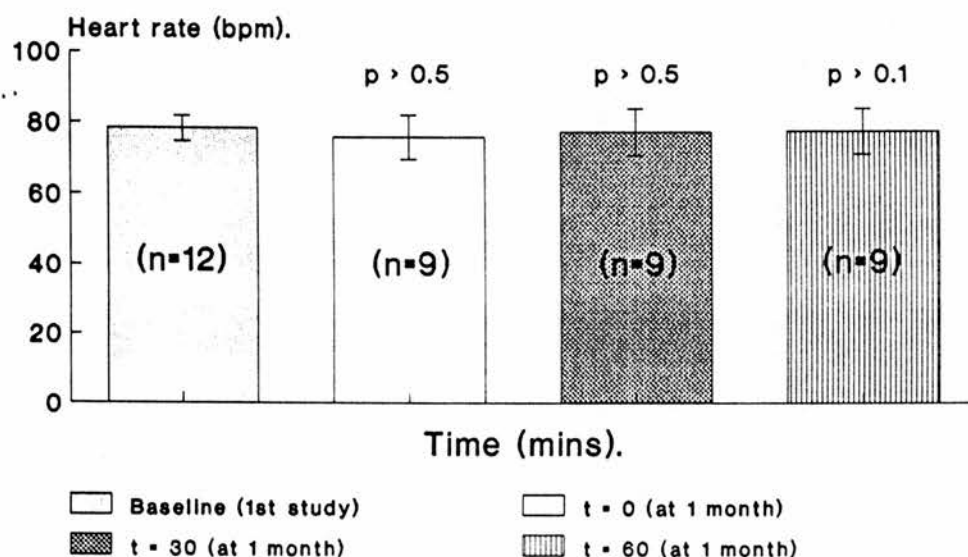


Figure 9

The mean heart rate response to chronic administration of 10mg Is-5-Mn in 12 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values from the first study.

The number of measurements possible for technical or attendance reasons is included in brackets.

Heart rate.

40mg Is-5-Mn - first study.

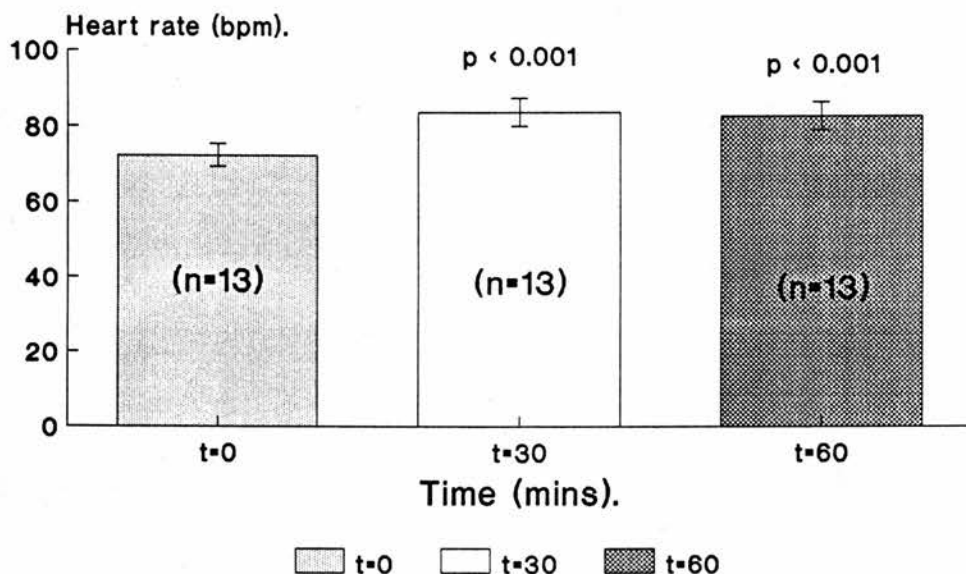


Figure 10

The mean heart rate response to acute administration of 40mg Is-5-Mn in 13 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values at the start of the study.

The number of measurements possible for technical or attendance reasons is included in brackets.

Heart rate. 40mg Is-5-Mn after 1 month.

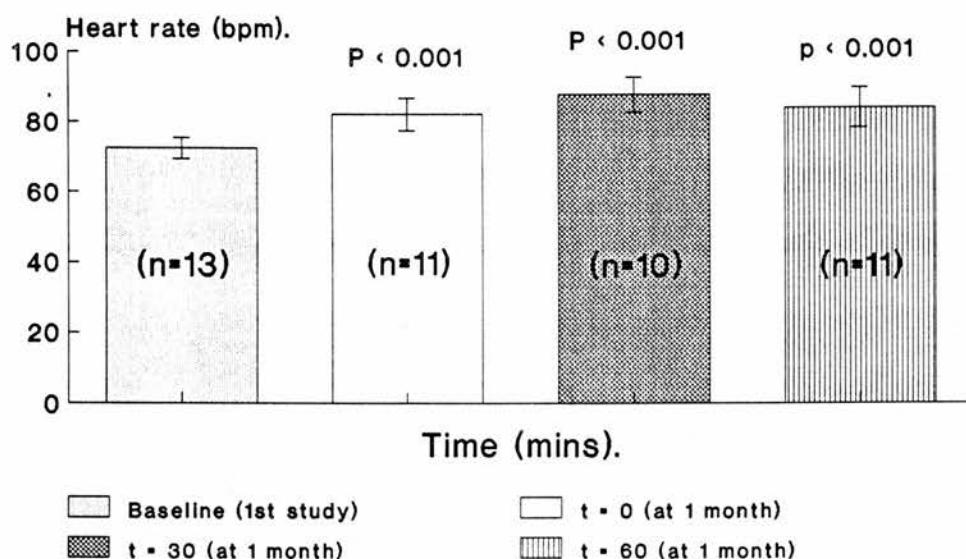


Figure 11

The mean heart rate response to chronic administration of 40mg Is-5-Mn in 13 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values from the first study.

The number of measurements possible for technical or attendance reasons is included in brackets.

Mean arterial blood pressure. 10 mg Is-5-Mn - first study.

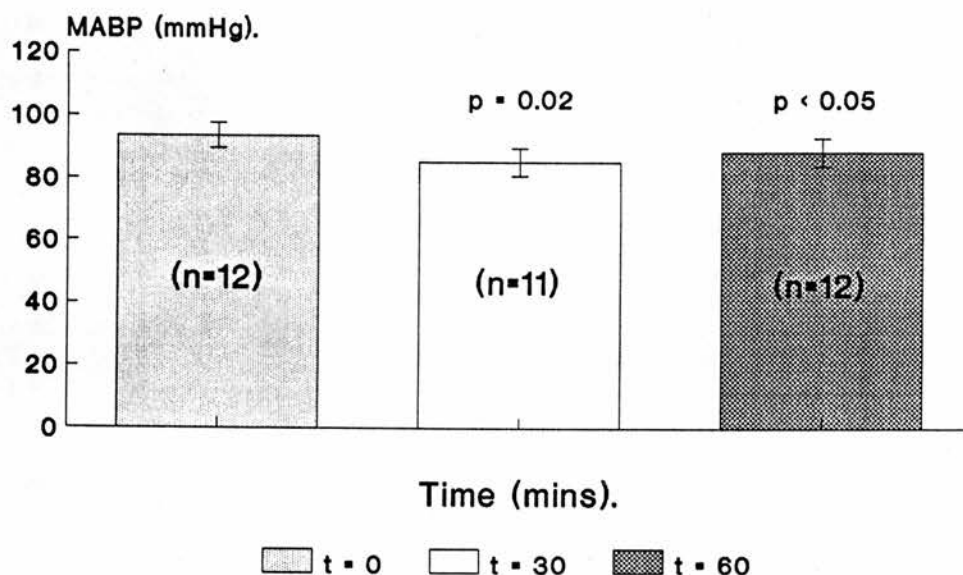


Figure 12

The mean arterial blood pressure response to acute administration of 10mg Is-5-Mn in 12 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values at the start of the study.

The number of measurements possible for technical or attendance reasons is included in brackets.

Mean arterial blood pressure. 10mg Is-5-Mn after 1 month.

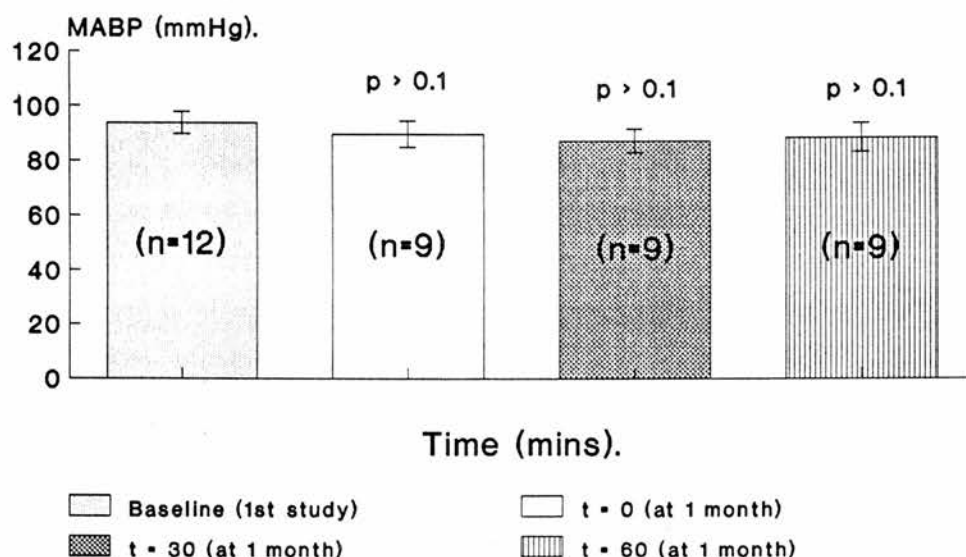


Figure 13

The mean arterial blood pressure response to chronic administration of 10mg Is-5-Mn in 12 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values from the first study.

The number of measurements possible for technical or attendance reasons is included in brackets.

Mean arterial blood pressure.

40mg Is-5-Mn - first study.

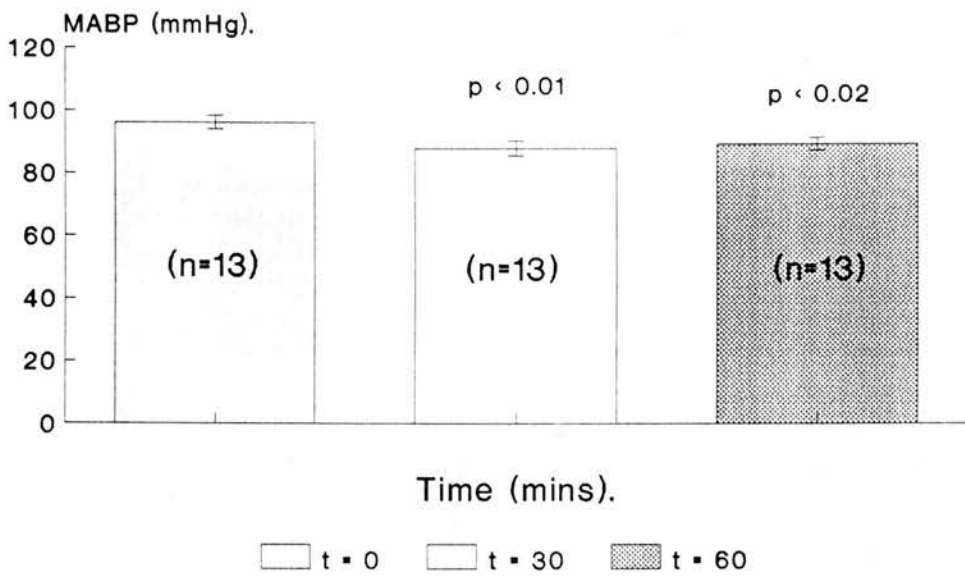


Figure 14

The mean arterial blood pressure response to acute administration of 40mg Is-5-Mn in 13 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values at the start of the study.

The number of measurements possible for technical or attendance reasons is included in brackets.

Mean arterial blood pressure. 40mg Is-5-Mn after 1 month.

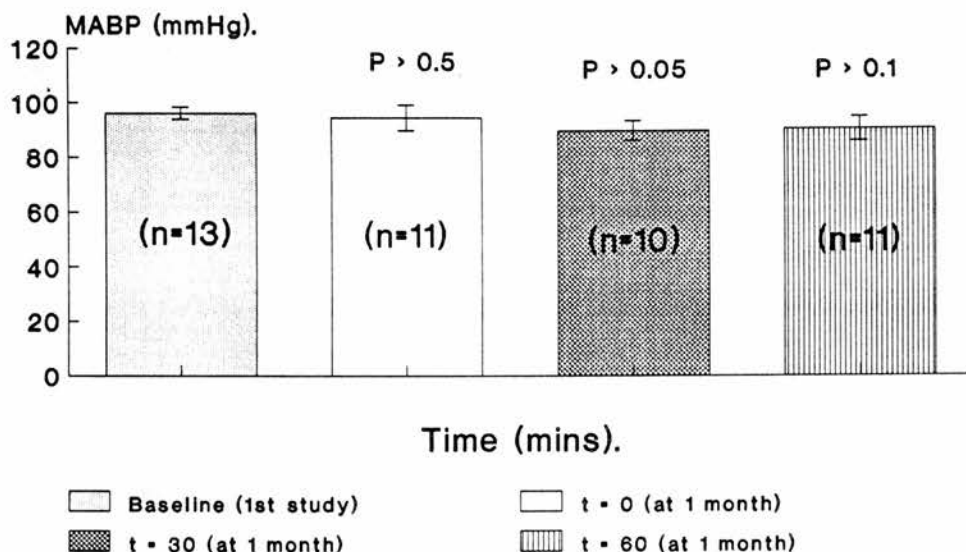


Figure 15

The mean arterial blood pressure response to chronic administration of 40mg Is-5-Mn in 13 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values from the first study.

The number of measurements possible for technical or attendance reasons is included in brackets.

Free hepatic venous pressure.
10mg Is-5-Mn.

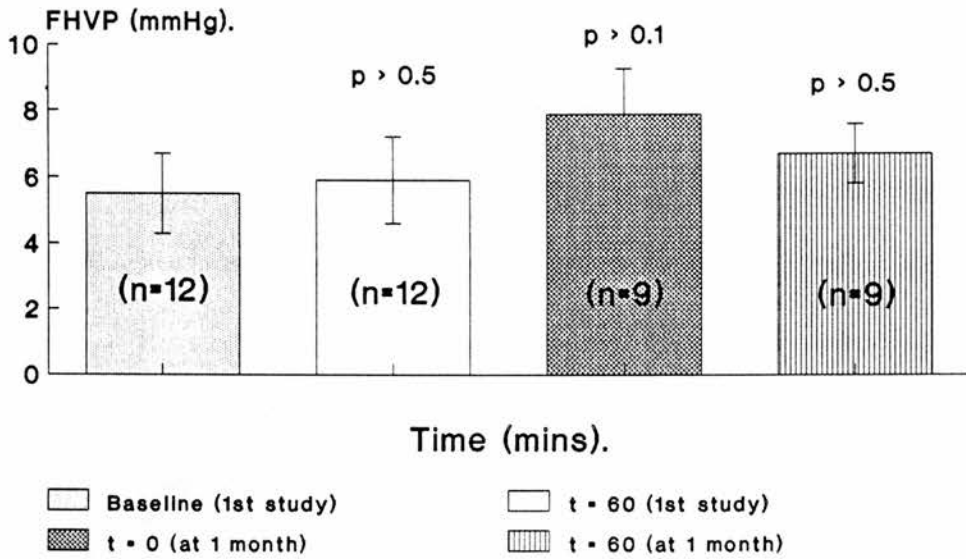


Figure 16

The mean FHVP response to acute and chronic administration of 10mg Is-5-Mn in 12 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values at the start of the study.

The number of measurements possible for technical or attendance reasons is included in brackets.

significantly 60 minutes after 10mg Is-5-Mn given initially and after 1 month of nitrate use (Figure 17). The pre-rechallenge pressure at 1 month was however not significantly different from the baseline (1st study) value. The hepatic venous pressure however gradient showed sustained reduction after acute and chronic nitrate use (Figure 18).

40mg Is-5-Mn had no statistically significant action on free hepatic venous pressure (Figure 19). Wedged hepatic venous pressure fell significantly acutely and after one month of 40mg Is-5-Mn (Figure 20), an increasing effect being seen with more exposure to the nitrate. The hepatic venous pressure gradient showed sustained reduction after acute and chronic nitrate use (Figure 21), the greatest effect being seen on rechallenge with nitrate after a month of therapy.

Wedge hepatic venous pressure 10mg Is-5-Mn.

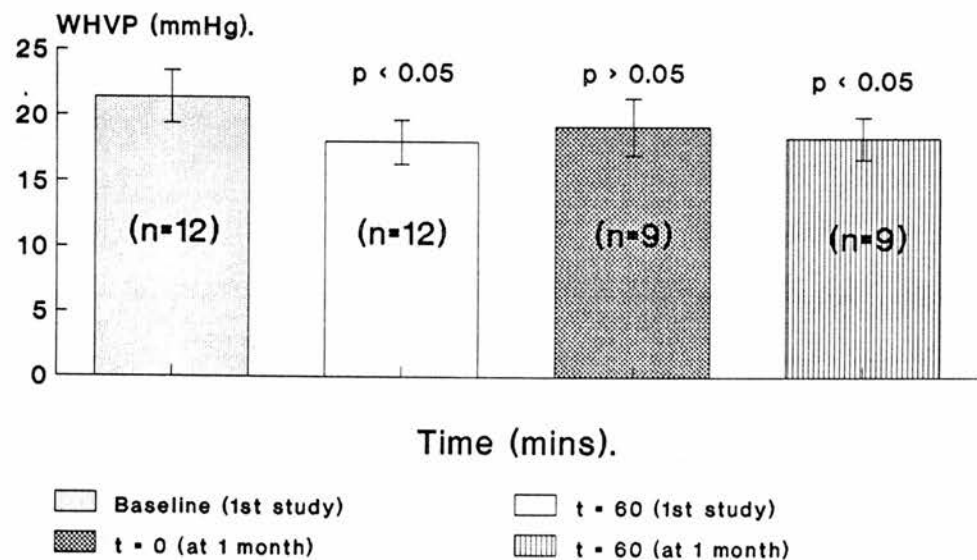


Figure 17

The mean WHVP response to acute and chronic administration of 10mg Is-5-Mn in 12 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values at the start of the study.

The number of measurements possible for technical or attendance reasons is included in brackets.

Hepatic venous pressure gradient 10mg Is-5-Mn.

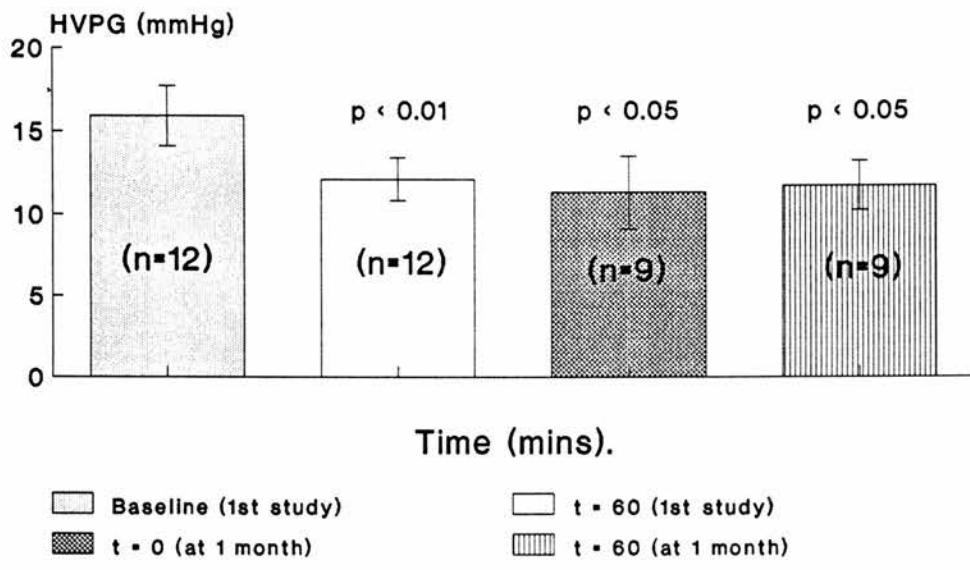


Figure 18

The mean hepatic venous pressure gradient changes in response to acute and chronic administration of 10mg Is-5-Mn in 12 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values at the start of the study.

The number of measurements possible for technical or attendance reasons is included in brackets.

Free hepatic venous pressure. 40mg Is-5-Mn.

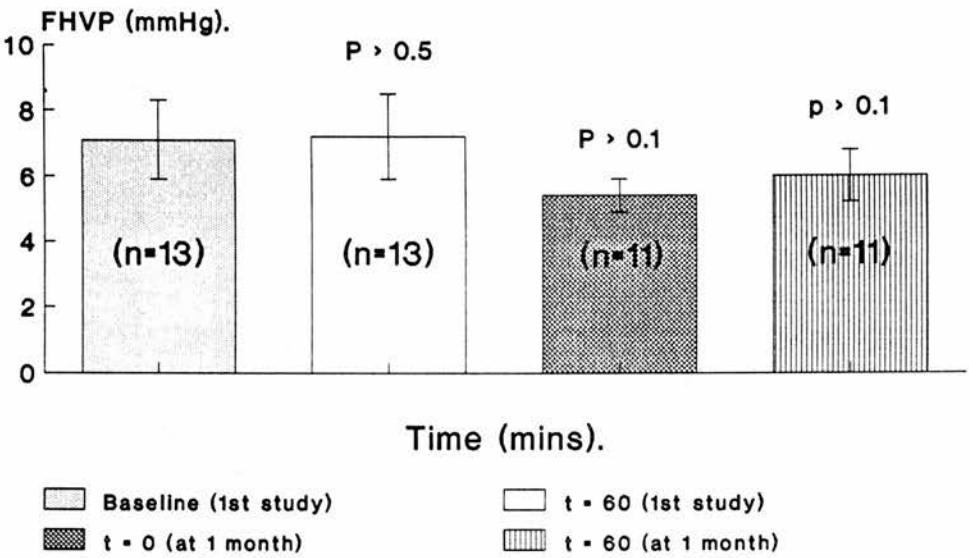


Figure 19

The mean FHVP response to acute and chronic administration of 40mg Is-5-Mn in 13 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values at the start of the study.

The number of measurements possible for technical or attendance reasons is included in brackets.

Wedged hepatic venous pressure 40mg Is-5-Mn.

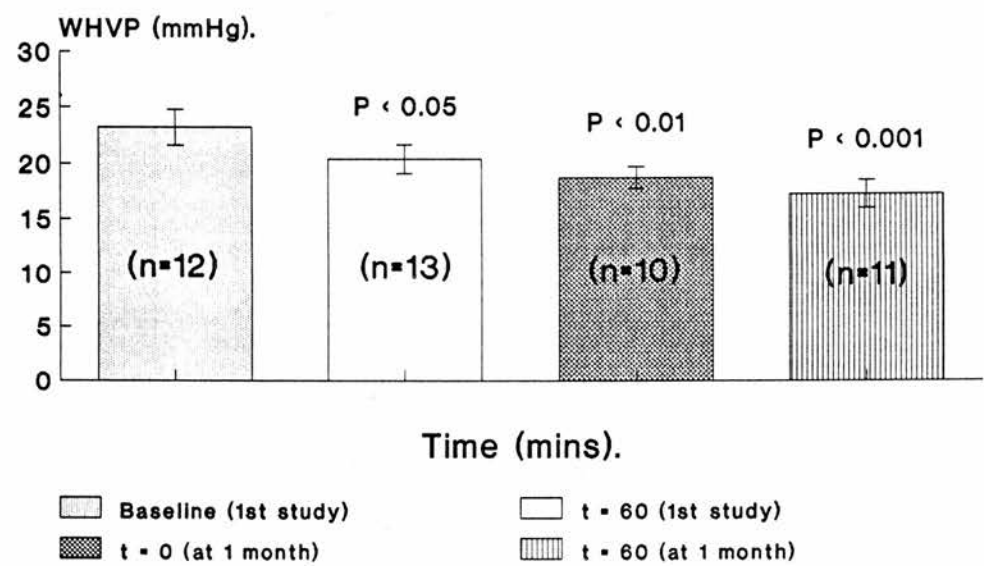


Figure 20

The mean WHVP response to acute and chronic administration of 40mg Is-5-Mn in 13 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values at the start of the study.

The number of measurements possible for technical or attendance reasons is included in brackets.

Hepatic venous pressure gradient 40mg Is-5-Mn.

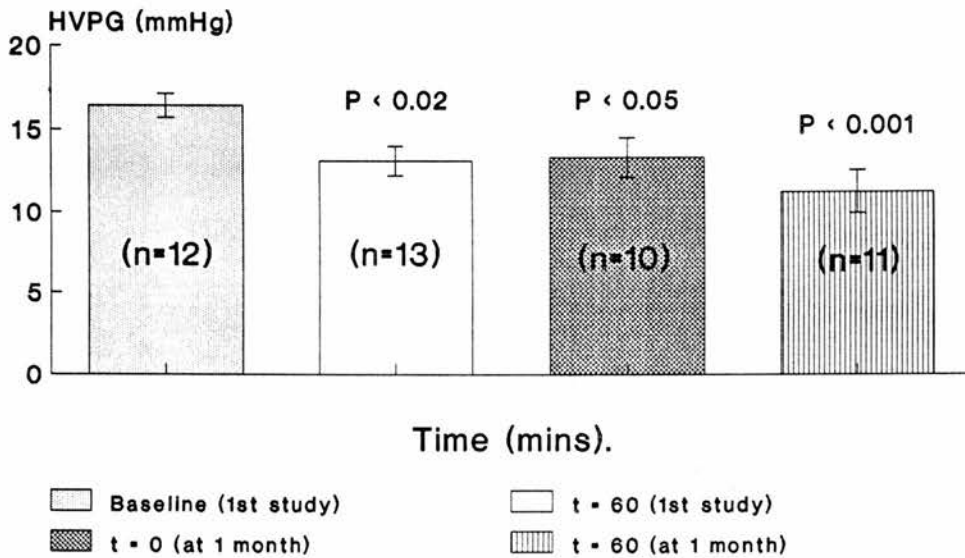


Figure 21

The mean HVPG response to acute and chronic administration of 40mg Is-5-Mn in 13 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values at the start of the study.

The number of measurements possible for technical or attendance reasons is included in brackets.

The effect of Is-5-Mn on azygos vein blood flow

10mg Is-5-Mn administration produced a significant reduction of azygos flow within thirty minutes of administration but thereafter no significant reduction was seen (Figure 22 and 23). Small reductions of azygos flow were seen acutely (Figure 24) and chronically (Figure 25) after 40mg Is-5-Mn use but these failed to reach statistical significance.

Most noticeable however was the marked variability of response of azygos blood flow to either dose of the nitrate as illustrated by the large standard error values in Figures 22-25. This can be explained by division of all 25 patients (i.e. both dosage groups) into arbitrary groups at baseline with low (less than 350 ml/min, moderate (350-550 ml/min) and high azygos flows (more than 550 ml/min). Although no statistical analysis between the flow groups would be valid because of the post hoc nature of the group allocation, Figure 26 shows that those with low initial values increase in response to nitrate and those with high initial values reduce in response to nitrate. Those with intermediate values demonstrate little change in response to nitrate.

Correlation between Childs'-Pugh variables and response to Is-5-Mn

(Spearman's' Rank Correlation Test)

No significant correlation was found between change in heart rate, mean arterial blood pressure, wedged hepatic venous pressure, free hepatic venous pressure, hepatic venous pressure gradient and azygos blood flow with serum bilirubin, albumin and prothrombin ratio in response to 10 mg Is-5-Mn at any time point in the first study or at 1 month (Appendices VII and VIII).

Azygos vein blood flow. 10mg Is-5-Mn - first study.

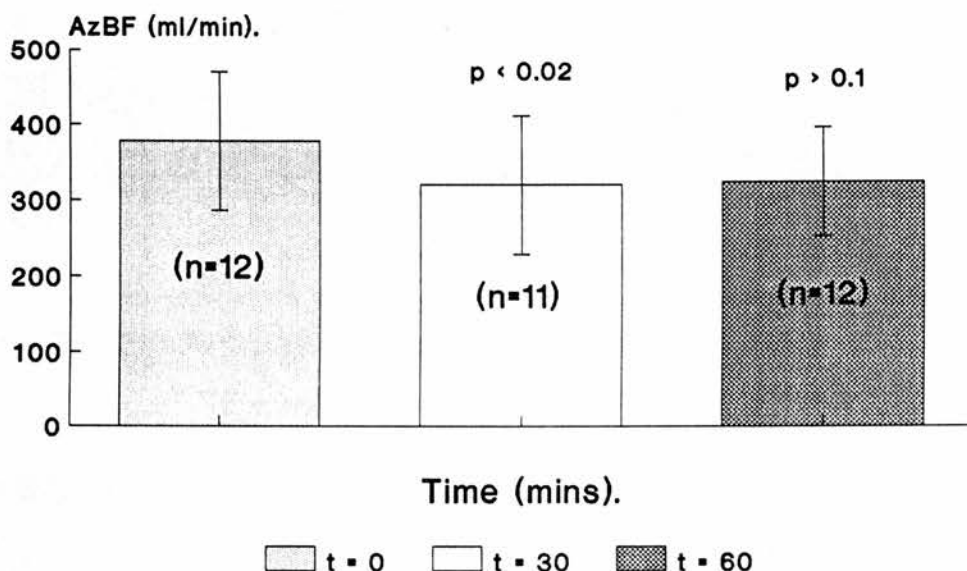


Figure 22

The mean azygos blood flow response to acute administration of 10mg Is-5-Mn in 12 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values at the start of the study.

The number of measurements possible for technical or attendance reasons is included in brackets.

Azygos vein blood flow. 10mg Is-5-Mn after 1 month.

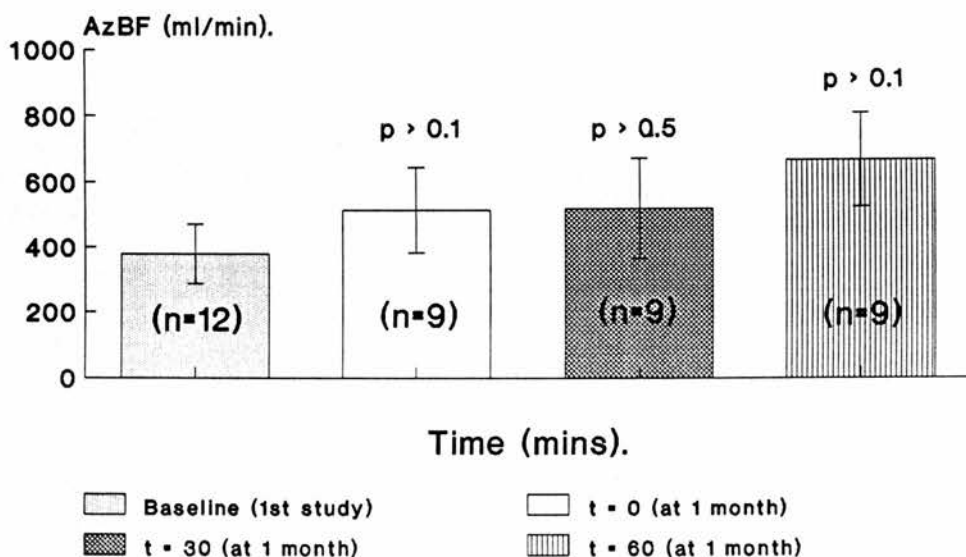


Figure 23

The mean azygos blood flow response to chronic administration of 10mg Is-5-Mn in 12 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values from the first study.

The number of measurements possible for technical or attendance reasons is included in brackets.

Azygos vein blood flow. 40mg Is-5-Mn - first study.

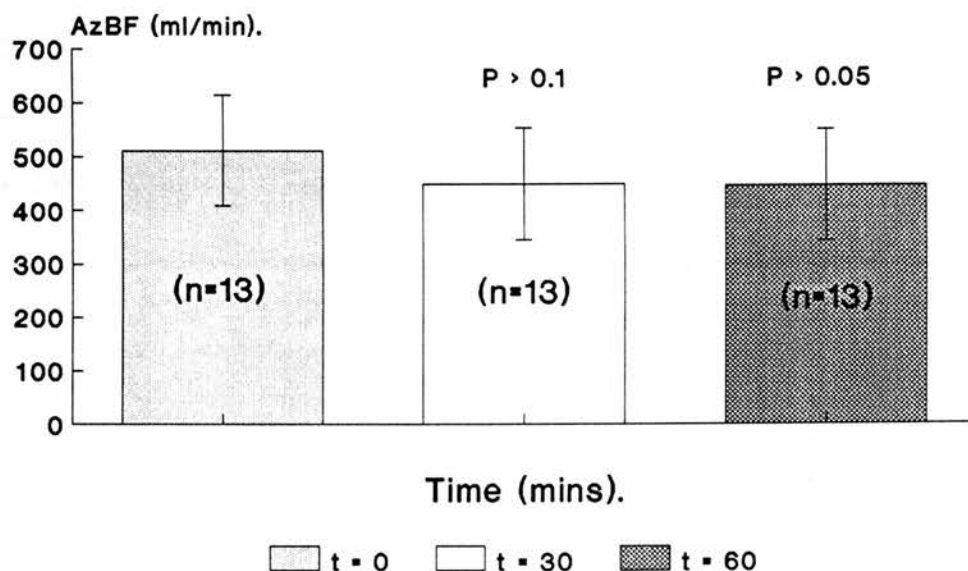


Figure 24

The mean azygos blood flow response to acute administration of 40mg Is-5-Mn in 13 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values at the start of the study.

The number of measurements possible for technical or attendance reasons is included in brackets.

Azygos vein blood flow. 40mg Is-5-Mn after 1 month.

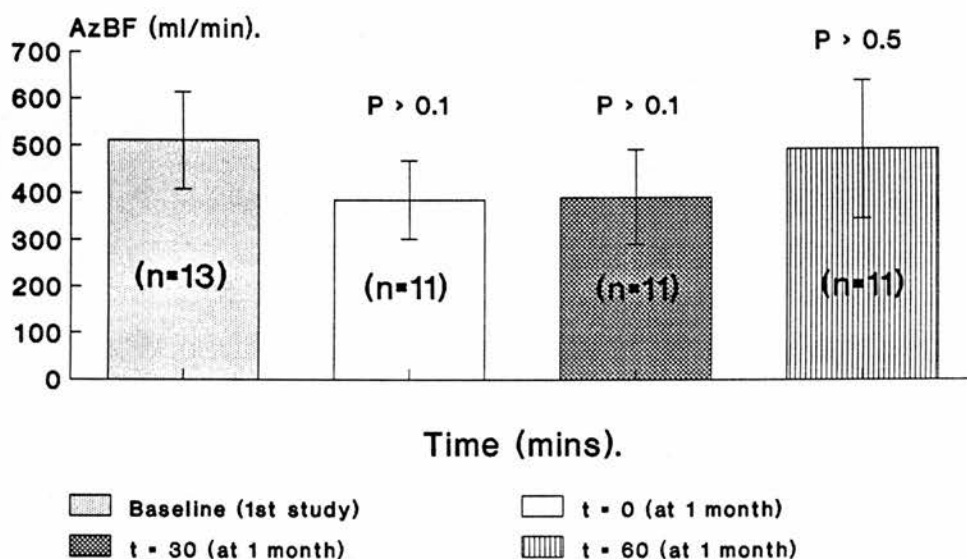


Figure 25

The mean azygos blood flow response to chronic administration of 40mg Is-5-Mn in 13 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values at the start of the study.

The number of measurements possible for technical or attendance reasons is included in brackets.

Mean azygos flow - response to Is-5-Mn.

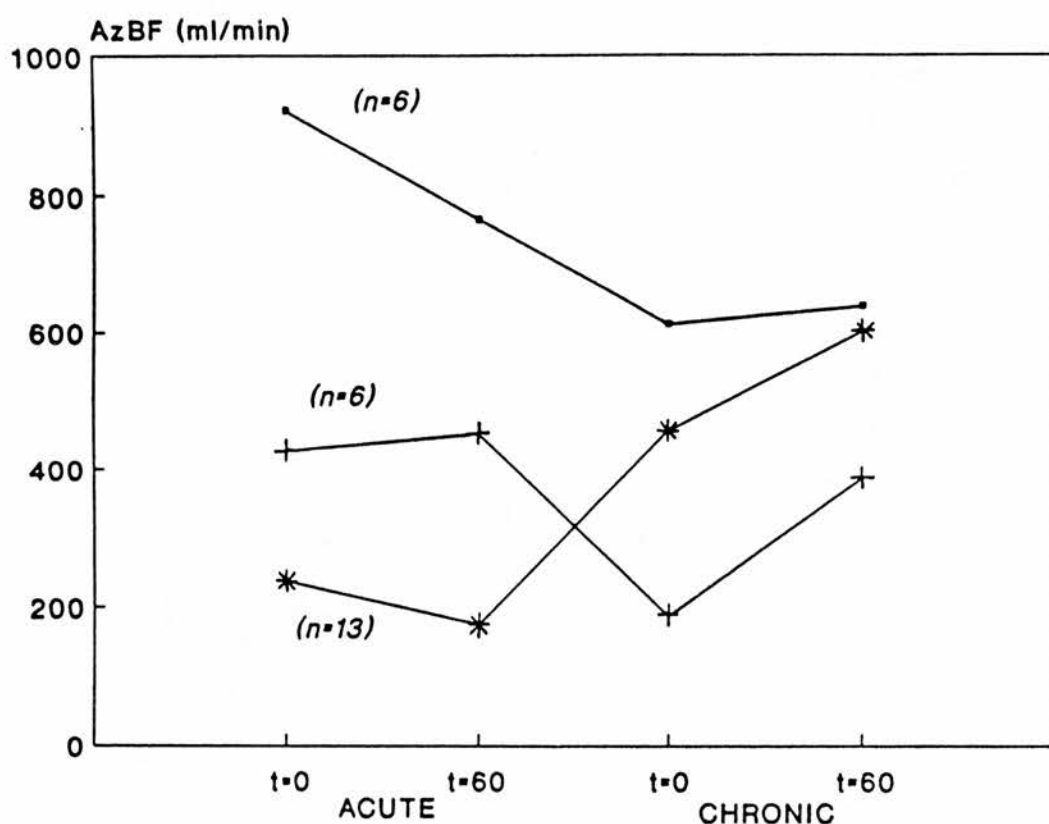


Figure 26

The mean azygos blood flow response to acute and chronic administration of Is-5-Mn (10mg & 40mg groups combined) in 25 patients with cirrhosis.

Patients are arbitrarily divided into three groups - those with:

- low initial azygos flows ($< 350\text{ml/min}$)
- moderate azygos flows ($350\text{-}550\text{ml/min}$)
- and high azygos flows ($> 550\text{ ml/min}$)

Prothrombin ratio and serum bilirubin were also found to be positively correlated in this group (0.775) (Appendices VII and VIII).

Similarly no correlations were found with the 40 mg group of Is-5-Mn except reduction of free hepatic venous pressure (in the first hour, first study, $r = -0.72$) and reduction of azygos blood flow (in the first hour, second study, $r = 0.750$) were both significantly correlated ($p < 0.05$) with serum bilirubin (Appendices IX and X). Also the reduction in wedged hepatic venous pressure at the end of the second study (at 1 month) was correlated with serum albumin ($r = 0.65$) (Appendices IX and X). Azygos blood flow reduction from baseline to time zero on the first and second study were both correlated with serum albumin ($r = -0.633$ and -0.667 , respectively). Of the haemodynamic variables measured only the reduction in free hepatic venous pressure after the first hour, first study correlated with the prothrombin time ratio ($r = 0.70$) in the 40 mg group (Appendices IX and X).

In the 40 mg group serum albumin correlated with Prothrombin ratio (-0.692) and serum bilirubin (0.717) but there was no significant correlation between serum albumin and serum alanine aminotransferase (0.326) (Appendices IX and X). Prothrombin time correlated with serum bilirubin in this group (0.650) (Appendices IX and X).

The effect of Is-5-Mn on estimated liver blood flow (ICG method)

Figures 27 and 28 show that only 40mg Is-5-Mn produces a significant fall in liver blood flow after one hour (which coincides with a drop in mean arterial

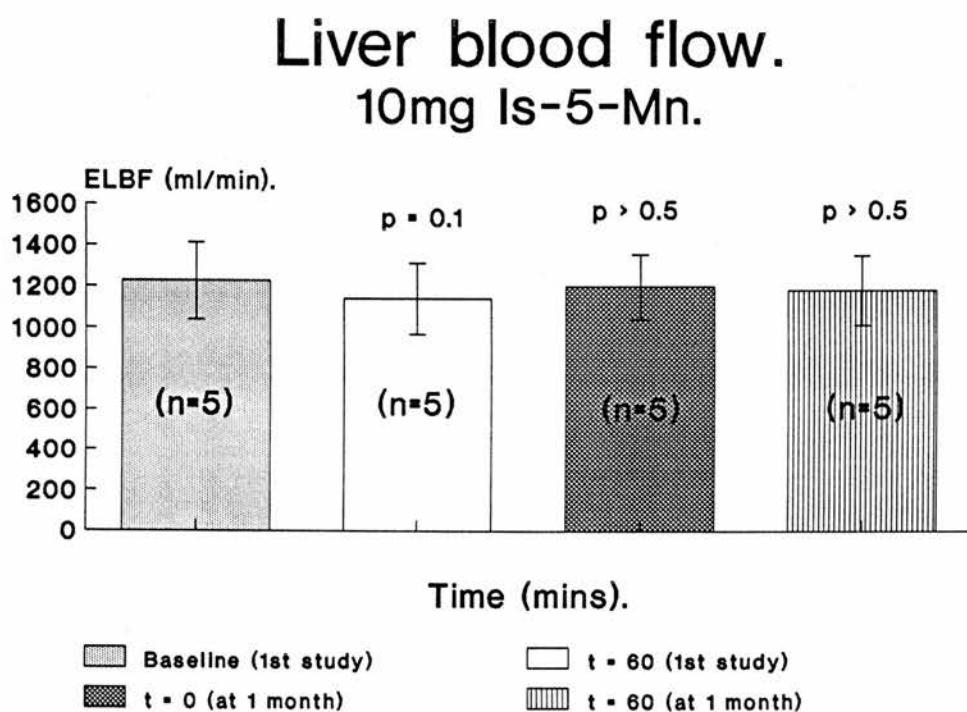


Figure 27

The mean estimated liver blood flow (ICG constant infusion method) after acute and chronic administration of 10mg Is-5-Mn in 5 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values at the start of the study.

Liver blood flow. 40mg Is-5-Mn.

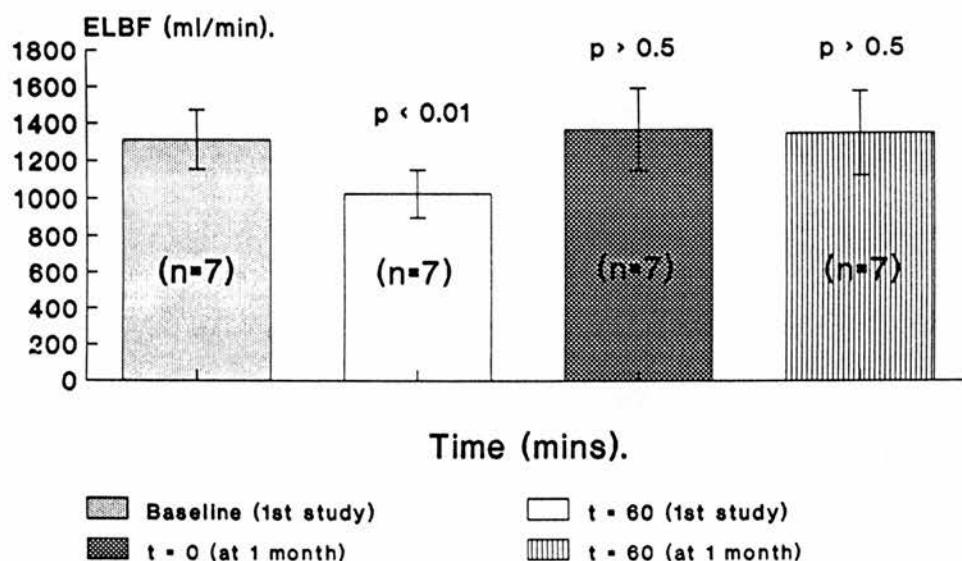


Figure 28

The mean estimated liver blood flow (ICG constant infusion method) after acute and chronic administration of 40mg Is-5-Mn in 7 patients with cirrhosis.

Error bars represent the standard error of the mean. Statistical comparison is made by paired t-test between values at each time point and the baseline values at the start of the study.

blood pressure). Liver blood flow did not show a significant reduction with chronic nitrate use.

Side-effects

3 patients receiving 10mg Is-5-Mn bd reported headaches during the first 24 hours of therapy (patient numbers 1, 2 and 7). One patient described persistent headaches (patient number 8) over the first two weeks and withdrew from the study.

Five patients reported initial headaches after starting 40mg Is-5-Mn which passed within 24 hours of starting them (patient numbers 1, 4, 10, 12, 13). One of these five patients experienced headaches for 10 days after commencing the tablets lasting 10-20 minutes after each tablet and withdrew from the study.

One patient (7) had an itchy maculopapular rash following the initial haemodynamic study. The nitrate tablets were withdrawn and recommenced 1 week later with no recurrence of the rash. We can only assume that either the indocyanine green dye or Cefotaxime were responsible. Both were omitted on the repeat study after one full month of nitrate tablets and no recurrence of the rash was seen.

Compliance

10 of the 10 mg group of patients (100% of those attending the follow-up haemodynamic study after 1 month) returned their unused tablets. 9 patients (90%) had returned the correct number of tablets and one patient had returned

one tablet too many. All claimed to have taken their nitrate tablets diligently and had outpatient appointment attendance rates in excess of 85%.

9 of the 40 mg group of patients (81.8% of those attending the follow-up haemodynamic study after 1 month) returned their unused tablets. 8 patients (72.7%) had returned the correct number of tablets and the remaining patient had three tablets too many. Again all patients claimed to have taken their nitrate tablets and had outpatient appointment attendance rates in excess of 90%.

PRELIMINARY DISCUSSION

No evidence for dose-dependency of Isosorbide-5-mononitrate in reduction of the hepatic venous pressure gradient

Our study suggests that both 10 mg and 40 mg of Is-5-Mn greatly reduce the hepatic venous pressure gradient acutely and with chronic use. This is of clinical relevance given the increasing interest in the use of nitrates to prevent variceal bleeding in cirrhotic patients.

Only two previous groups have shown no effect of nitrates on the hepatic venous pressure gradient (Dawson et al, 1985; Tsai et al, 1989). All others showed a reduction in hepatic venous pressure gradient which was accompanied by a fall in cardiac index and mean arterial blood pressure (Isosorbide dinitrate (Hallemans et al, 1983; Freeman et al, 1985; Merkel et al, 1987; Blei et al, 1987; Qureshi et al, 1988; Mols et al, 1989, Is-5-Mn: Hayes et al, 1988; Navasa et al, 1989). In our study there was no apparent advantage of one dose over the other in achieving HVPG reduction.

How is the reduction in hepatic venous pressure gradient achieved?

The reduction of hepatic venous pressure gradient in previous studies and ours (by both doses of Is-5-Mn) was achieved by a fall in wedged hepatic venous pressure rather than a rise in free hepatic venous pressure.

Reducing wedged hepatic venous pressure may be more important than reducing the hepatic venous pressure gradient per se in protection against variceal bleeds (Bosch, 1985; Bosch et al, 1988a).

The evidence for different doses working by different mechanisms

Many haemodynamic studies to date suggest that different doses of nitrate may act by different mechanisms (Navasa et al, 1989; Rector et al, 1990) and this may well be the case in our study. Certainly the 40 mg dose appears to induce a greater haemodynamic disturbance acutely ie. increased heart rate, reduced mean arterial blood pressure and liver blood flow than the 10 mg dose; only the increase in heart rate effect was maintained after chronic use. Whether this translates into greater efficacy of the higher dose in prophylaxis of variceal bleeding remains to be evaluated by clinical studies.

Heterogeneity in collateral vessel responsiveness to nitrates

Efficacy of drugs in controlling an acute variceal bleed or preventing recurrent haemorrhage may be related to their ability to reduce blood flow through collateral vessels, thus determination of the effects of vasoactive agents on both collateral blood flow and the hepatic venous pressure gradient is important in evaluating their therapeutic potential.

Our results indicate that the effect of 10 mg or 40 mg Is-5-Mn on azygos blood flow was highly variable including some individuals in who, flow increased suggesting vasodilatation of portasystemic collaterals. Those who had a low azygos flow increased their flow in response to nitrate and vice-versa. Thus the degree of pre-existing porta-systemic shunting appears important in the individuals' response to nitrate. Although the azygos flow tends to increase in those with more advanced liver disease we did not observe a clear correlation between Childs-Pugh grade (1973) and haemodynamic response (see below).

The role of severity of liver disease in predicting an individuals' response to nitrate

Severity of liver disease per se does not appear an important variable in defining haemodynamic response to nitrates as so few haemodynamic changes correlated with serum bilirubin, albumin or PTR (which are the best available, though imperfect measures of severity). A few correlations were positive but this would be expected by chance alone given the large number of correlations tested.

Other variables that have previously been suggested to be responsible for different response to nitrates between individuals may be degree of autonomic impairment (Moreau et al, 1989) or baseline cardiac filling pressure (Rector et al, 1990).

Tolerance to nitrates

Tolerance is well recognised in patients with heart disease given nitrates for 18 hours or longer (Elkayam et al, 1987; Jugdutt and Warnica, 1989). Our study and others (Freeman et al, 1985; Cervinka et al, 1989; Tsai et al, 1989; Garcia-Pagan et al, 1990; Ikegami et al, 1992; Vorobioff et al, 1992) showed a sustained fall in hepatic venous pressure gradient after chronic nitrate administration which suggests full tolerance is not of concern in patients with chronic liver disease. To postulate why this may be the case we need to first consider possible mechanisms of tolerance and these will be discussed later (Section VI).

The effect of nitrates on liver blood flow

Calculations of hepatic resistance from previous studies described above and our own study shows that acute and chronic administration of nitrates induce both a significant reduction in resistance rather than a reduction in liver blood flow (which would be undesirable). This implies that either portal flow is not reduced or that a compensatory rise in hepatic arterial flow takes place ie. reciprocity. This mechanism, although observable in animals, probably has little effect in man and an increase in hepatic arterial flow would be unlikely in view of the fall in mean arterial blood pressure and cardiac index caused by nitrates (Hayes et al, 1988). The difficulty in distinguishing between these two inputs limits our ability to interpret this data further.

Compliance and side-effects

Our data suggest that the patients were taking their nitrate tablets during this study; their motivation to attend outpatient clinics was high and the discrepancy of tablet counts minimal. Side-effects were modest.

Concluding remarks

Our work suggests that chronic administration of either 10 or 40 mg of Is-5-Mn, allowing for a nitrate free interval each day is effective at achieving hepatic venous pressure gradient reduction but has a variable effect on blood flow that appears to depend on baseline azygos flow. Unfortunately no noninvasive means of assessing the latter is currently available.

Nitrates potentially have a number of different mechanisms of hepatic venous pressure reduction which may vary at different doses. Fortunately, by mechanisms which remain to be elucidated, patients with cirrhosis do not develop full tolerance to nitrates in the dosing regimen used in our studies.

Section III

The effect of N-acetylcysteine on systemic and portal haemodynamics and oxygen delivery and consumption in patients with cirrhosis

INTRODUCTION

Administration of intravenous N-acetylcysteine (NAC) has been reported to increase cardiac output, tissue oxygen delivery and utilisation in fulminant hepatic failure (Harrison et al, 1991). Since some of the haemodynamic changes seen in patients with cirrhosis are similar to those with fulminant hepatic failure, N-acetylcysteine may also increase tissue oxygen use and delivery in patients with cirrhosis. The aim of this study was to determine the haemodynamic effects of this agent in cirrhosis with particular reference to oxygen delivery and consumption.

MATERIALS AND METHODS

The population studied

11 biopsy proven cirrhotic patients (8 men; 3 women; mean age 61.5 years; range 43-74 years) with a range of disease severity (4A, 2B, 5C Child-Pugh grades (Pugh et al, 1973) and aetiology (2 primary biliary, 1 cryptogenic and 8 alcohol) were studied (Appendix XI). Exclusion criteria included known myocardial infarction or ischaemic heart disease, valvular heart disease, pregnancy, vasoactive medication, current viral hepatitis B,C or D or bleeding diathesis with prothrombin time ratio (PTR) greater than 2.5:1.

The eight alcoholic cirrhotic patients had no evidence of alcoholic cardiomyopathy.

All patients gave witnessed informed consent and ethical permission was obtained from Lothian Health Board Medical Ethics Subcommittee.

Investigations prior to study

Routine investigations prior to undertaking each study included estimation of serum urea and electrolytes, a full blood count including haematocrit, "liver function tests" including bilirubin, alanine aminotransferase (ALT), gamma-glutamyl peptidase (gamma-GT) and albumin (Appendix XI). Each patient had a prothrombin time estimated prior to the invasive procedure (Appendix XI).

The study procedure

Each patient fasted after a light breakfast on the day of the study. In the catheter laboratory a 7.5 F introducer (Edwards, Critical Care Division, Irvine, USA) was placed in the right femoral vein under local anaesthesia (approx. 10 ml of 2% lignocaine). A Swan-Ganz catheter (7F Edwards, Irvine, USA) was inserted under fluoroscopic screening and continuous pressure recording into the pulmonary artery. The tip of the catheter was positioned in a major branch of the pulmonary artery. The mean heart rate (from the ECG monitor, Hewlett Packard HP monitoring system, Germany) and mean arterial blood pressure (manual syphygmomanometer) were checked every 5 minutes initially until all patients had achieved haemodynamic stability for at least 20 minutes.

Simultaneous femoral artery, pulmonary artery and femoral vein blood gas samples were then taken into heparinised blood gas syringes and analysed immediately for oxygen saturation (Co-Oximeter 282, Instrumentation Laboratory, Lexington, Mass.), haemoglobin and oxygen tension (pO_2) (ABL 2000, Radiometer, Copenhagen). The pulmonary artery free and wedge

pressure and cardiac output (by Swan-Ganz thermodilution method using 10 ml of cold 5% dextrose as injectate) were estimated. Cardiac output was measured in quadruplicate and the results expressed as a mean value. Cardiovascular pressures were measured with reference to the mid-axillary line.

N-acetylcysteine (Parvolex, Duncan Flockhart, Greenford, UK) was infused intravenously by accurate infusion pump (Gemini 2, IVAC, USA) at 150 mg/Kg in 200 ml 5% dextrose over 15 minutes followed by 15 minutes infusion at 125 ml/hour of 50 mg/Kg in 500 ml 5% dextrose, ie identical regime to that of Harrison and colleagues (1991).

The blood gas analysis, pulmonary artery pressures, mean arterial blood pressure, cardiac output and heart rate were remeasured 30 minutes after commencement of N-acetylcysteine (NAC) infusion i.e. during infusion.

Derived haemodynamic variables for each patient were calculated according to standard formulae (Packer et al, 1985; Appendix XII).

The delivery of oxygen to tissues was calculated as the product of the cardiac index and the arterial oxygen content. Oxygen consumption was calculated from the reverse Fick equation (cardiac index \times a-v O₂ difference) (Bihari, 1993). The oxygen extraction ratio was calculated by dividing the difference between the arterial and venous O₂ content by the arterial O₂ content and expressed as a percentage.

The hepatic venous pressure gradient (wedged hepatic venous pressure minus free hepatic venous pressure) and estimated liver blood flow (ICG, Cardiogreen, Hynson, Westcott and Dunning Inc, Baltimore, Md - continuous infusion method (Cherrick et al, 1960; Winkler et al, 1965) were measured immediately before and after NAC infusion in 6 of the 11 patients with cirrhosis (the last 6 patients studied) using the methods described in Section II - materials and methods. The intravenous infusion of indocyanine green (ICG) was made up in saline and infused at a rate of 0.25 mg/min after a priming dose of 0.20 mg/Kg/ body weight.

Controls

As the systemic haemodynamic effect of this infusion regime of NAC has been assessed previously in patients without liver disease it was felt that inclusion of normal healthy controls in an invasive study such as this was unethical (Packer et al, 1987; Winniford et al, 1986).

The haemodynamic response to infusion of the same volume of 5% dextrose as above but not containing N-acetylcysteine on a random allocation basis (random numbers) to 5 of the above 11 patients with cirrhosis (marked by a * in Appendix XI) was assessed to investigate any potential volume loading effect of the dextrose infusion on cardiac output etc, ie 11 patients with cirrhosis took part in these studies; all 11 received the NAC infusion (made up in 5% dextrose); in 5 of the 11 patients (controls) the effect of dextrose infusion alone was also studied.

Statistical analysis of haemodynamic variables

The results after the infusion of NAC were compared with the baseline haemodynamic values before infusion. All results were expressed as mean \pm SEM with statistical analysis by a 2-tailed paired student's t-test.

RESULTS

Administration of N-acetylcysteine had no effect on mean arterial blood pressure (88 ± 2.7 mmHg before NAC to 85.8 ± 3.3 mmHg after NAC; $p > 0.1$ (Figure 29)) or mean heart rate (78.3 ± 3.2 bpm before NAC to 79.2 ± 2.68 bpm after NAC; $p > 0.05$ (Figure 30)).

The infusion of NAC resulted in vasodilatation with a significant reduction in both mean systemic vascular resistance index (1902 ± 195 to 1645 ± 193 dynes \times sec/cm⁵ \times m²; $p < 0.02$ (Figure 31)) and pulmonary vascular resistance index (169.2 ± 28 to 127 ± 21.0 dynes \times sec/cm⁵ \times m²; $p < 0.01$ (Figure 32)).

Mean estimated liver blood flow increased from 1161 (± 167) ml/min to 1283 (± 92) ml/min in response to NAC infusion; $p > 0.1$ but this did not achieve statistical significance (Figure 33). Wedged hepatic venous pressure, free hepatic venous pressure and HVPG showed no significant change after N-acetylcysteine infusion (Table 12).

Administration of NAC resulted in a significant increase in mean oxygen delivery (from 1086 ± 167 ml/min to 1214 ± 140 ml/min; $p < 0.01$ (Figure

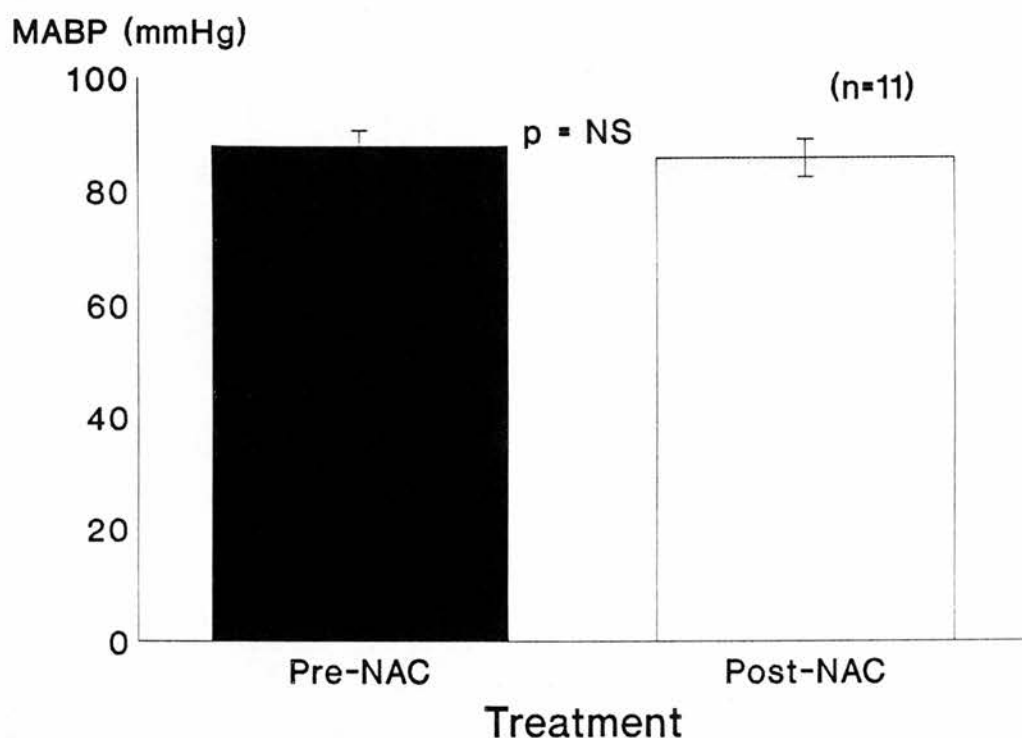


Figure 29

The effect of N-acetylcysteine infusion (NAC) at 150 mg/kg body weight for 15 minutes in 200 ml 5% dextrose followed by 15 minutes of 50 mg/kg in 500 ml 5% dextrose (set at 125 ml/hour) on mean arterial blood pressure (MABP) in 11 patients with cirrhosis.

The vertical bars represent mean values and the lines above the bars the standard error of the mean.

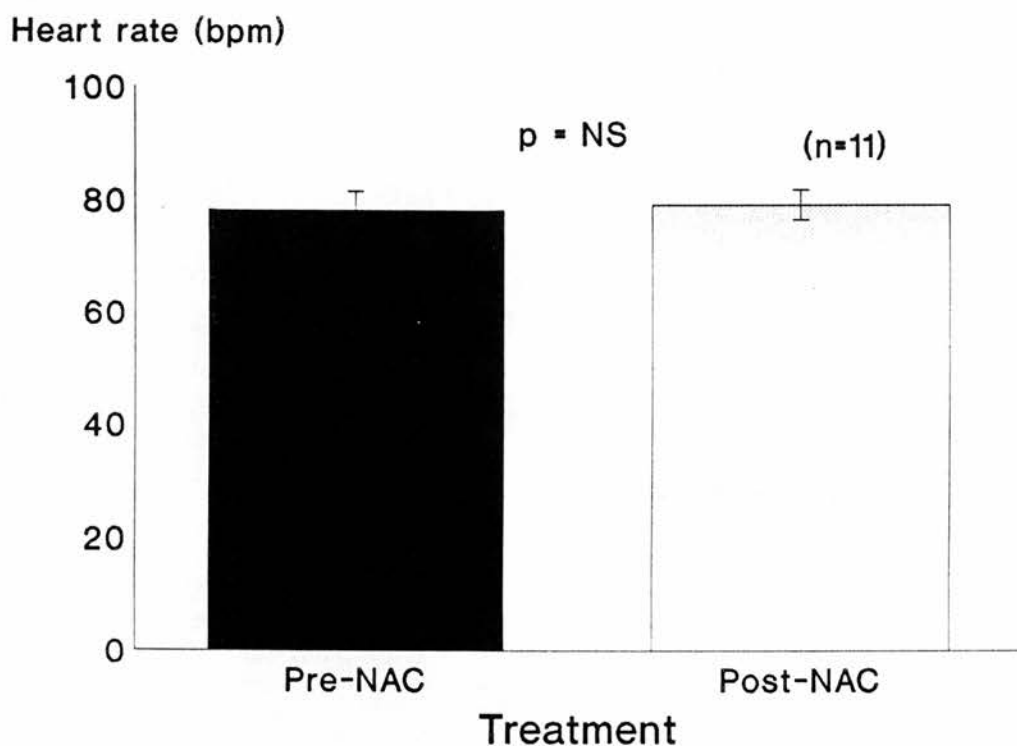


Figure 30

The effect of N-acetylcysteine infusion (NAC) at 150 mg/kg body weight for 15 minutes in 200 ml 5% dextrose followed by 15 minutes of 50 mg/kg in 500 ml 5% dextrose (set at 125 ml/hour) on mean heart rate in 11 patients with cirrhosis.

The vertical bars represent mean values and the lines above the bars the standard error of the mean.

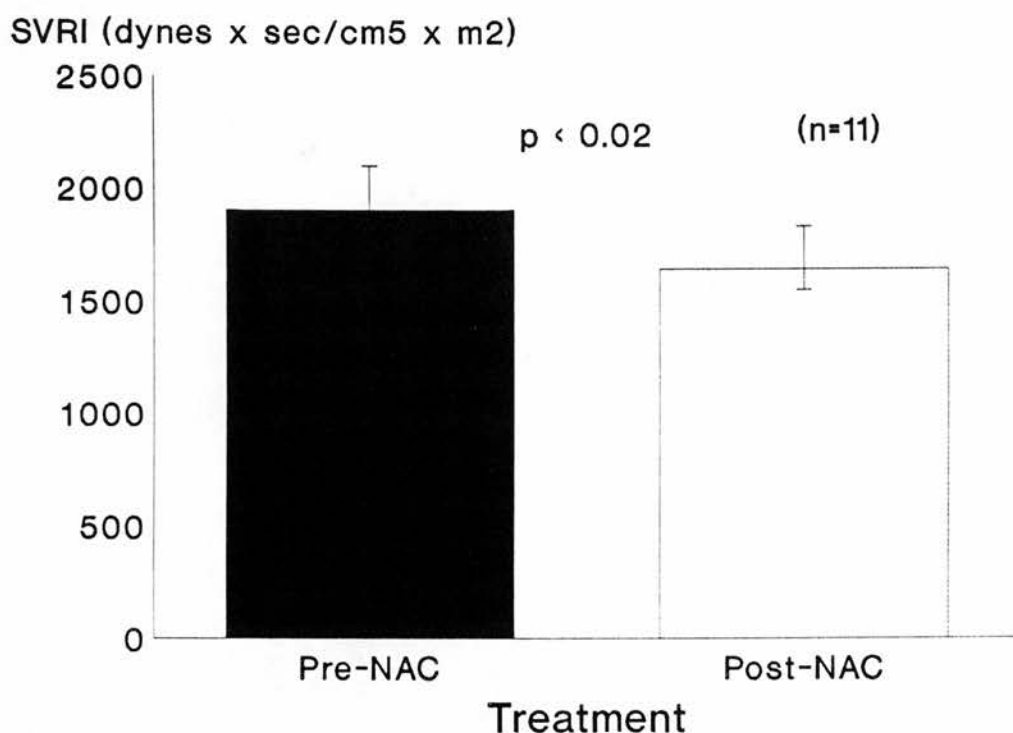


Figure 31

The effect of N-acetylcysteine infusion (NAC) at 150 mg/kg body weight for 15 minutes in 200 ml 5% dextrose followed by 15 minutes of 50 mg/kg in 500 ml 5% dextrose (set at 125 ml/hour) on mean systemic vascular resistance index (SVRI) in 11 patients with cirrhosis.

The vertical bars represent mean values and the lines above the bars the standard error of the mean.

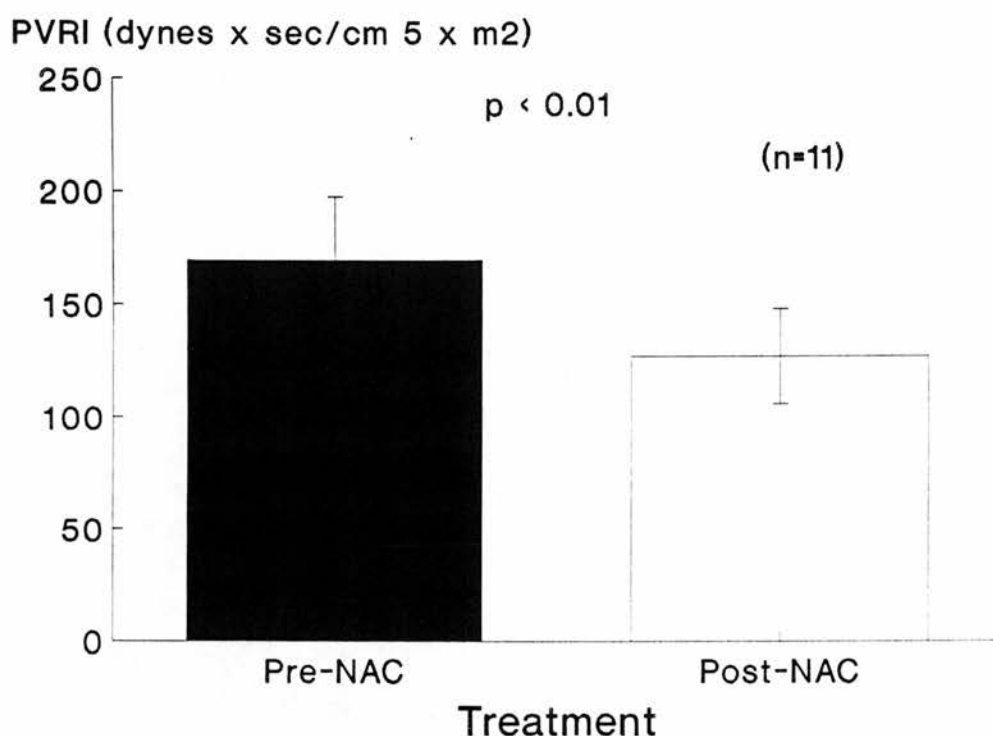


Figure 32

The effect of N-acetylcysteine infusion (NAC) at 150 mg/kg body weight for 15 minutes in 200 ml 5% dextrose followed by 15 minutes of 50 mg/kg in 500 ml 5% dextrose (set at 125 ml/hour) on mean pulmonary vascular resistance index (PVRI) in 11 patients with cirrhosis.

The vertical bars represent mean values and the lines above the bars the standard error of the mean.

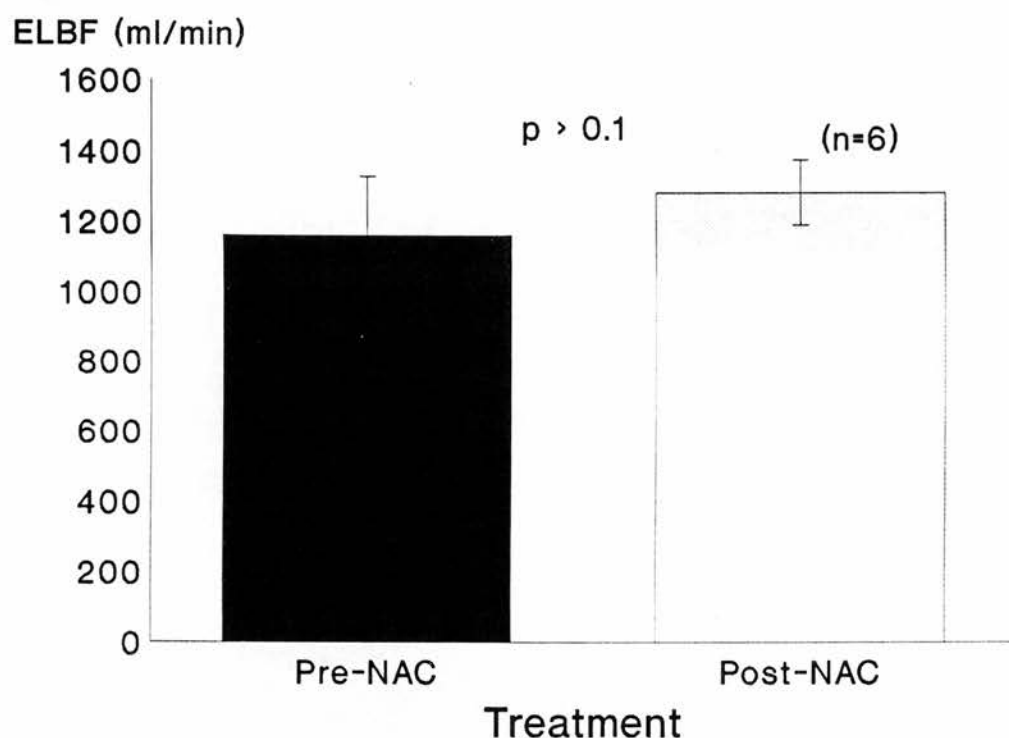


Figure 33

The effect of N-acetylcysteine infusion (NAC) at 150 mg/kg body weight for 15 minutes in 200 ml 5% dextrose followed by 15 minutes of 50 mg/kg in 500 ml 5% dextrose (set at 125 ml/hour) on estimated liver blood flow (ELBF) in 6 patients with cirrhosis.

The vertical bars represent mean values and the lines above the bars the standard error of the mean.

	Pres-NAC	Post-NAC
Wedged hepatic venous pressure (mmHg)	20.7	22.2 p = NS
Free hepatic venous pressure (mmHg)	7.3	8.5 p = NS
Hepatic venous pressure gradient (mmHg)	13.3	13.7 p = NS

Table 12

The effect of N-acetylcysteine infusion (NAC) at 150 mg/kg body weight for 15 minutes in 200 ml 5% dextrose followed by 15 minutes of 50 mg/kg in 500 ml 5% dextrose (set at 125 ml/hour) on wedged and free hepatic venous pressure in 6 patients with cirrhosis.

Significance is taken at the 95% level.

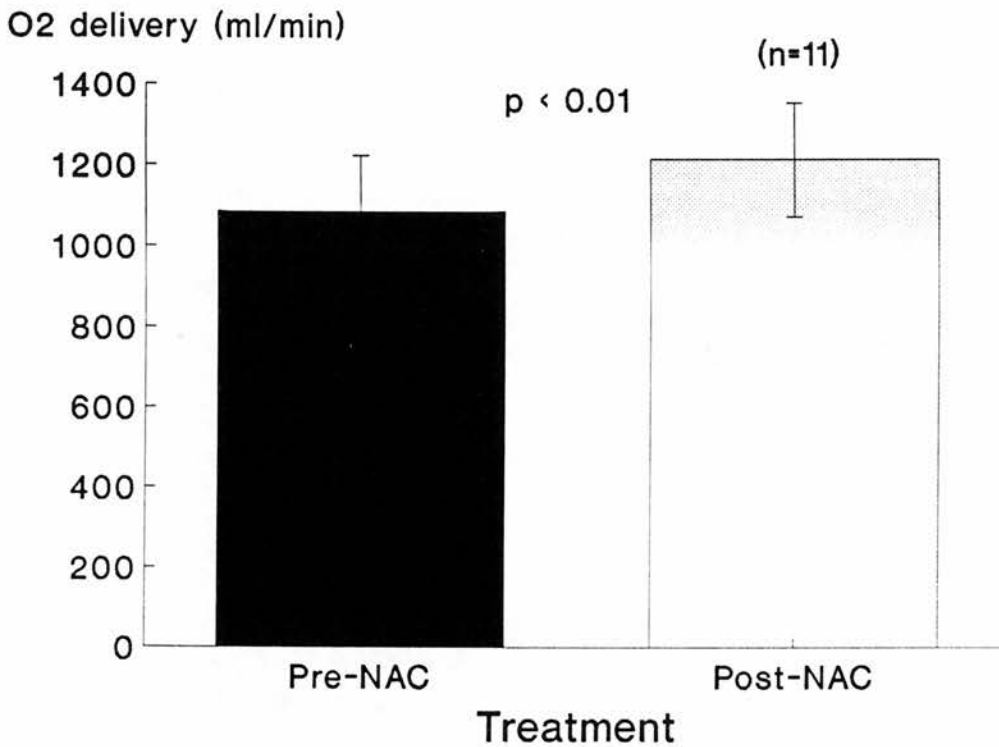


Figure 34

The effect of N-acetylcysteine infusion (NAC) at 150 mg/kg body weight for 15 minutes in 200 ml 5% dextrose followed by 15 minutes of 50 mg/kg in 500 ml 5% dextrose (set at 125 ml/hour) on mean oxygen delivery in 11 patients with cirrhosis.

The vertical bars represent mean values and the lines above the bars the standard error of the mean.

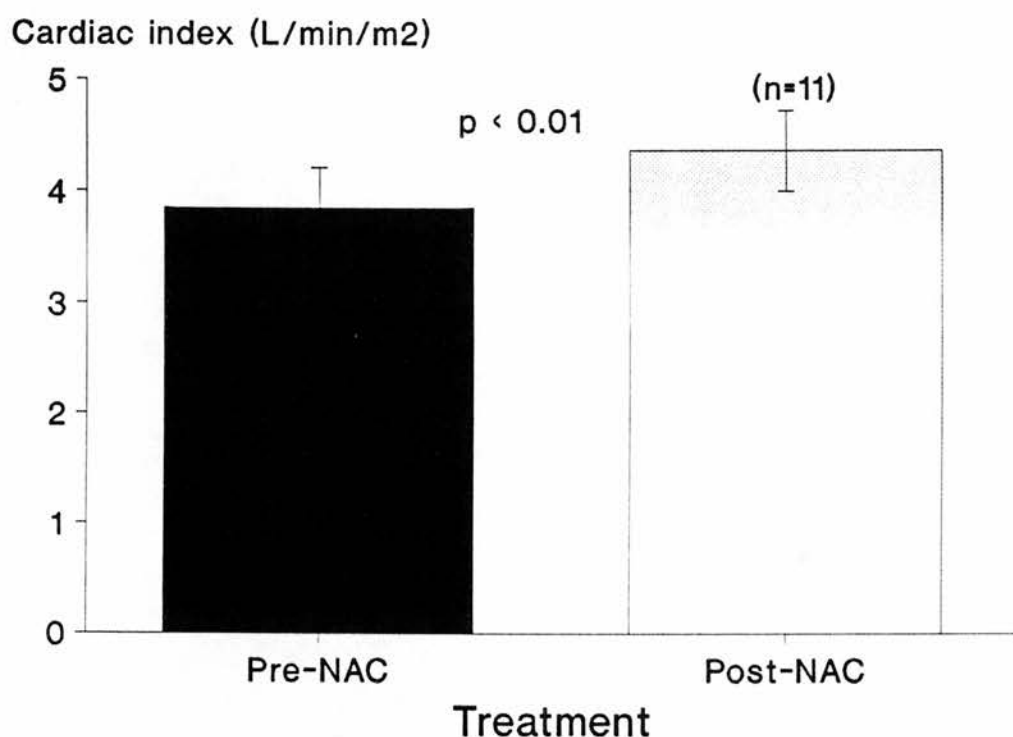


Figure 35

The effect of N-acetylcysteine infusion (NAC) at 150 mg/kg body weight for 15 minutes in 200 ml 5% dextrose followed by 15 minutes of 50 mg/kg in 500 ml 5% dextrose (set at 125 ml/hour) on mean cardiac index in 11 patients with cirrhosis.

The vertical bars represent mean values and the lines above the bars the standard error of the mean.

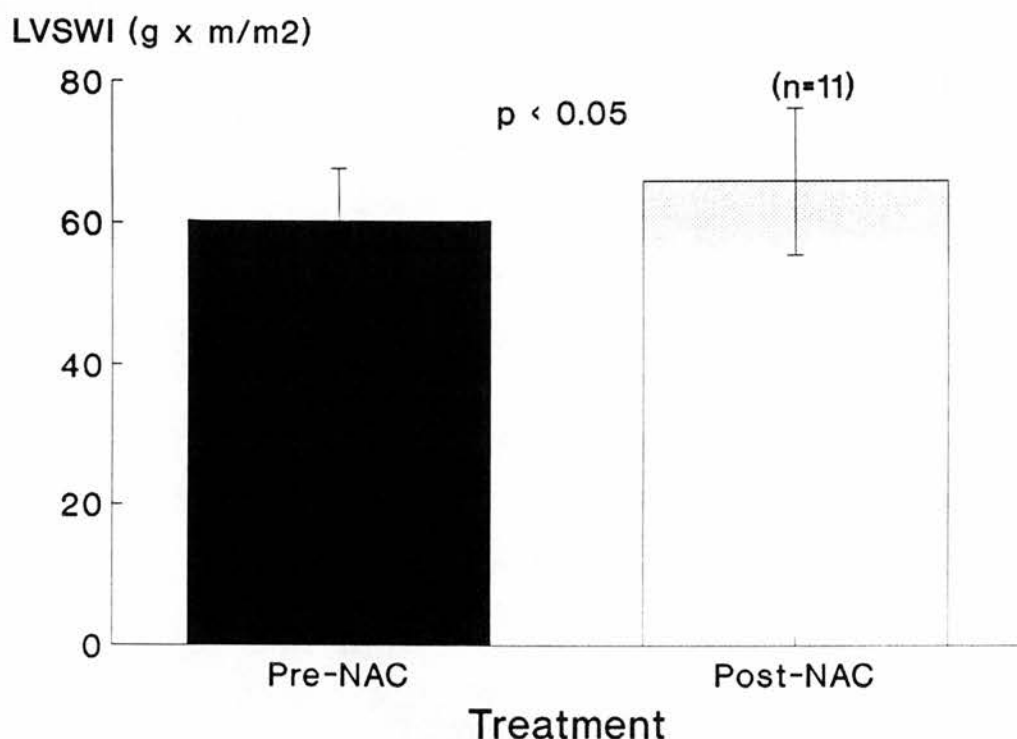


Figure 36

The effect of N-acetylcysteine infusion (NAC) at 150 mg/kg body weight for 15 minutes in 200 ml 5% dextrose followed by 15 minutes of 50 mg/kg in 500 ml 5% dextrose (set at 125 ml/hour) on left ventricular stroke work index in 11 patients with cirrhosis.

The vertical bars represent mean values and the lines above the bars the standard error of the mean.

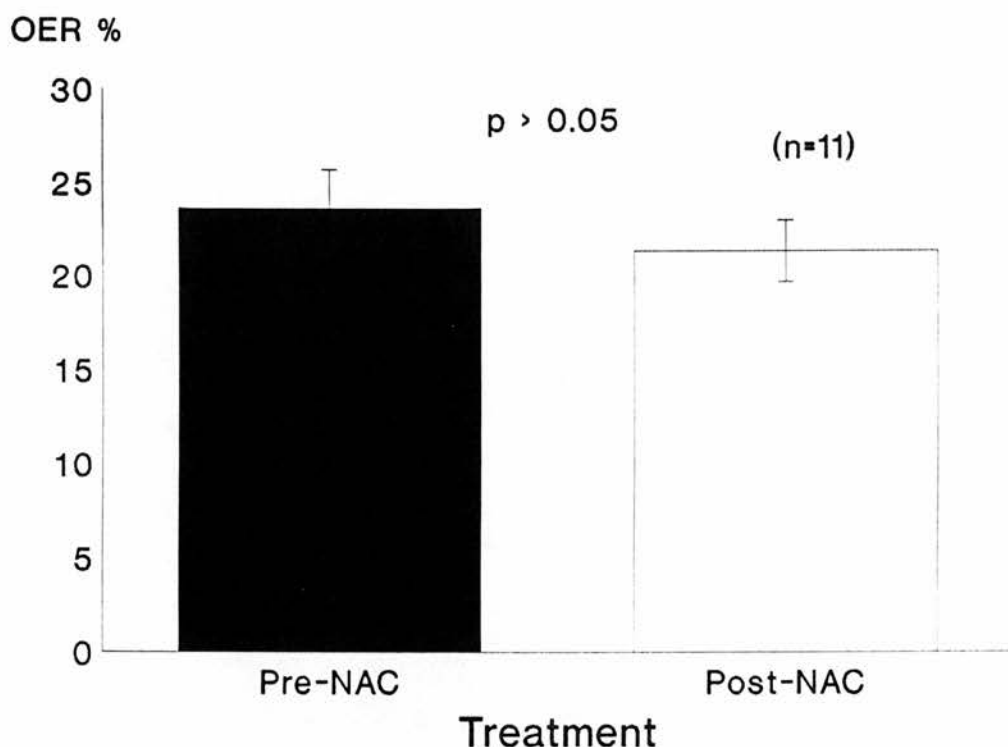


Figure 37

The effect of N-acetylcysteine infusion (NAC) at 150 mg/kg body weight for 15 minutes in 200 ml 5% dextrose followed by 15 minutes of 50 mg/kg in 500 ml 5% dextrose (set at 125 ml/hour) on mean arteriovenous oxygen extraction ratio (OER) in 11 patients with cirrhosis.

The vertical bars represent mean values and the lines above the bars the standard error of the mean.

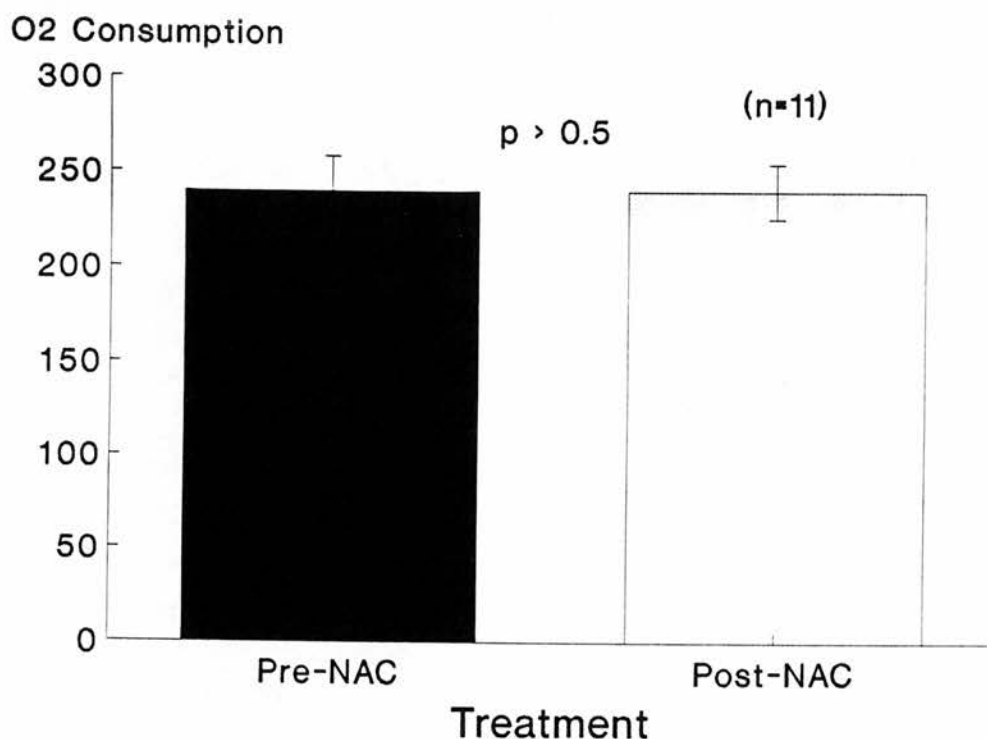


Figure 38

The effect of N-acetylcysteine infusion (NAC) at 150 mg/kg body weight for 15 minutes in 200 ml 5% dextrose followed by 15 minutes of 50 mg/kg in 500 ml 5% dextrose (set at 125 ml/hour) on mean oxygen consumption in 11 patients with cirrhosis.

The vertical bars represent mean values and the lines above the bars the standard error of the mean.

	Pre-NAC	Post-NAC	p
Femoral artery pO ₂ (kPa)	11.25	10.32	NS
Femoral artery O ₂ saturation (%)	94.3	93.7	NS
Pulmonary artery pO ₂ (kPa)	5.35	5.37	NS
Pulmonary artery O ₂ saturation (%)	72.1	73.8	NS
Femoral vein pO ₂ (kPa)	5.15	5.7	NS
Femoral vein O ₂ saturation (%)	67.9	74.9	NS

Table 13

The effect of N-acetylcysteine infusion (NAC) at 150 mg/kg body weight for 15 minutes in 200 ml 5% dextrose followed by 15 minutes of 50 mg/kg in 500 ml 5% dextrose (set at 125 ml/hour) on mean blood gas values in 11 cirrhotic patients.

Significance is taken at the 95% level.

34), due to an increase in the mean cardiac index from 3.85 ± 0.35 L/min/m² to 4.36 ± 0.36 L/min/m²; $p < 0.01$ (Figure 35)). This was associated with a significant increase in Left Ventricular Stroke Work Index from 60.3 ± 7.3 to 65.9 ± 10.3 g x m/m²; $p < 0.05$ (Figure 36).

However the arteriovenous oxygen extraction ratio did not rise significantly (23.6% to 21.3%; $p > 0.05$, Fig 37) after NAC administration. In addition the mean oxygen consumption did not rise in response to NAC (Fig 38).

The effect of N-acetylcysteine on mean blood gas values (Table 13) showed a minor fall in arterial oxygen tension and increase in venous oxygen tension after NAC administration.

The control group

Five patients who received intravenous dextrose alone showed no significant change in haemodynamic parameters (Table 14).

(CONTROL DATA: The haemodynamic response of five of the 11 patients with cirrhosis to 5% dextrose infusion alone)

	Baseline	After 5% dextrose alone	p value
Heart rate (bpm)	74.4 ± 4.6	73.2 ± 4.1	NS
MABP (mmHg)	91 ± 3.3	89.2 ± 3.1	NS
RAP (mmHg)	3.8 ± 0.37	3.8 ± 0.37	NS
CI (L/min/m ²)	3.46 ± 0.3	3.54 ± 0.26	NS
SVRI (dynes x sec/cm ⁵ x m ²)	2064 ± 184	2008 ± 183	NS
LVSWI (g x m/m ²)	59 ± 7.8	60.2 ± 5.3	NS
PVRI (dynes x sec/cm ⁵ x m ²)	188 ± 44	184.7 ± 48.5	NS

Table 14

Values are expressed as mean ± standard error of the mean. MABP is mean arterial blood pressure, RAP is right atrial pressure, CI is cardiac index, SVRI and PVRI are systemic and pulmonary vascular resistance index respectively. LVSWI is left ventricular stroke work index.

Significance is taken at the 95% level.

Spearman Rank Correlation Test

There was no significant statistical correlation between Child's variables (PTR, serum bilirubin and albumin) and degree of change in response to NAC of cardiac index, oxygen delivery, oxygen consumption and oxygen extraction ratio (Appendices XIII and XIV).

However serum bilirubin and rise in free hepatic venous pressure were positively correlated (0.833) and serum albumin and reduction of wedged hepatic venous pressure were correlated (0.683).

The prothrombin ratio correlated with reduced wedged hepatic venous pressure (-0.891) and also correlated with reduced radial artery pO_2 (0.794) and pulmonary artery pO_2 (0.809).

Amongst the haemodynamic variables correlations were found of right atrial pressure and reduction of pulmonary artery pressure (0.985), reduction of pulmonary capillary wedge pressure and reduction of radial artery pO_2 (0.746), reduction of pulmonary capillary wedge pressure and wedged hepatic venous pressure (-0.804), reduction of radial artery pO_2 and reduced wedged hepatic venous pressure (-0.891) and reduced pulmonary artery pO_2 and wedged hepatic venous pressure (-0.792) in response to infusion of N-acetylcysteine.

PRELIMINARY DISCUSSION

We have demonstrated that administration of NAC resulted in reduction of pulmonary and systemic vascular resistance in patients with cirrhosis similar to the effect seen in fulminant hepatic failure (Harrison et al, 1991). This effect has not been found in patients with cardiac failure, with symptoms of chest pain nor in those fully recovered from acute liver failure (Harrison et al, 1991; Packer et al, 1987; Winniford et al, 1986). The reduction in systemic vascular resistance may represent vasodilation. The reduction in pulmonary vascular resistance may be due to a variety of mechanisms including pulmonary vasodilation and shunting which are discussed further in Section VI (Rodriguez-Roisin et al, 1992).

The 5 patients in the control group showed no vasodilation in response to dextrose infusion alone. Thus the postulate that the haemodynamic changes in the 11 patients given N-acetylcysteine infusion were due to effects of volume loading or patients relaxing after the initial invasive procedures can be ruled out and the changes seen in patients given NAC infusions result from a drug effect.

In patients with cirrhosis, unlike those with fulminant hepatic failure (Harrison et al, 1991), the mean arterial pressure did not change (Figure 29), the oxygen extraction ratio (Figure 37) and O_2 consumption did not rise in response to NAC infusion (Figure 38). The cardiac index (Figure 35), systemic vascular resistance index (Figure 31), pulmonary vascular resistance index (Figure 32), stroke work index (Figure 36) and O_2 delivery (Figure 34) percentage changes

were similar in cirrhotic and fulminant hepatic failure patients (Harrison et al, 1991).

As most drugs that primarily cause afterload reduction increase cardiac index without affecting left ventricular stroke work index (or rate pressure product, or other measures of myocardial oxygen consumption) the rise in cardiac index in association with left ventricular stroke work index seen here is suggestive of a positive inotropic response rather than purely peripheral vasodilation and reduction in left ventricular afterload.

Although not statistically significant there was a consistent reduction in arterial pO_2 after NAC infusion in patients with cirrhosis (Table 13). As this was accompanied by a similar increase in O_2 saturation in mixed venous blood the combined effect was a decrease in the oxygen extraction ratio (OER) (Figure 37). Because the number of patients undertaking such an invasive study was small and the fall in OER was partially compensated by a substantial rise in cardiac index in each patient the fall in oxygen consumption failed to achieve statistical significance. Since the administration of NAC to our 11 patients had clearly conferred no haemodynamic benefit we felt that to study more patients would be unethical.

Interestingly, there were no reported changes in arterial pO_2 , calculated a-v tension gradient and shunt in hepatic failure patients in response to NAC (Harrison et al, 1991). Errors in estimation of mixed venous O_2 saturation and tension could occur if the blood is drawn rapidly or if the catheter is positioned

peripherally within the pulmonary artery. These errors result from contamination of the mixed venous pulmonary blood with arterialised blood drawn from the pulmonary capillaries and veins. We are confident that sufficient care was taken in both sampling and in placement of the Swan-Ganz catheter to prevent this error occurring in our study. This is supported by the increasing pO_2 trend seen in peripheral vein samples after NAC infusion, parallelling the increase in pulmonary artery pO_2 following the infusion (Table 13).

We believe that the difference between our findings of the effect of NAC on O_2 delivery and consumption in patients with cirrhosis from those of Harrison (et al, 1991) in patients with fulminant hepatic failure, may relate to pathophysiological differences between the two groups of patients (Rodriguez-Roisin et al, 1992), errors in the Swan-Ganz catheter technique that can occur under certain circumstances (Bartlett & Dechert, 1990) or the phenomenon of mathematical coupling (Archie, 1981). It is thus possible that NAC may in fact increase O_2 delivery but not O_2 consumption with fulminant hepatic failure, and early studies indicate this is the case (Walsh - personal communication).

The putative mechanism of vasodilatation is also controversial and discussed later but it should be appreciated that not all vasodilators have the same

haemodynamic effects in patients with cirrhosis i.e. nitrates cause vasodilatation but have been reported to cause a fall in liver blood flow, cardiac output and mean arterial blood pressure and an increase in systemic vascular resistance (Hayes et al, 1988); this presumably reflects different mechanisms or sites of action of vasodilating agents.

The splanchnic circulation seems to have been spared from the haemodynamic changes seen in the systemic circulation in that estimated liver blood flow remained unchanged. In addition, the lack of changes in WHVP, HVPG and FHVP presumably reflects this sparing as any dilatation unaccompanied by a reduction in pressure would be due to increased inflow (according to Ohm's law) and this was not observed. Therefore although the azygos blood flow was not measured in this study it may well not have changed.

There were few correlations noted between change in haemodynamic variables and serum albumin, bilirubin and prothrombin ratio. These may have occurred by chance alone as the number of variables tested by Spearman Rank Correlation was large. That several haemodynamic changes correlated with each other, for example, reduction of pulmonary artery pressure and right atrial pressure is expected.

The administration of N-acetylcysteine to patients with cirrhosis is unlikely to be of therapeutic value and may be detrimental. This study illustrates that extrapolation of drug use between patients with fulminant hepatic failure and chronic liver disease is not always justifiable.

Section IV

The effect of N-acetylcysteine on forearm blood flow

INTRODUCTION

As described in Section I, patients with cirrhosis of the liver have been shown to exhibit arterial vasodilatation and moderate systemic hypotension; the so-called hyperdynamic circulation of cirrhosis.

We have shown that systemic administration of N-acetylcysteine to such patients (Section III) caused a reduction in the systemic vascular resistance index and a marked increase in cardiac output.

We believe that the reduction of systemic vascular resistance index may be due to arteriolar dilatation. By assessing the effect of small doses of N-acetylcysteine (given locally) on blood flow in the forearm we hope to establish whether its action is on peripheral arterial blood vessels through, for example, relaxation of the vascular smooth muscle (rather than a central action which causes vasodilation). Thus our hypothesis is that N-acetylcysteine acts as a peripheral vasodilator in such patients.

	Patients with cirrhosis	Control subjects	p value
Number	6	6	-
Sex	4 male, 2 female	6 male	-
Mean age (years)	60.7 ± 2.5	56 ± 2.3	p>0.5
Age range (years)	55-69	47-62	-
MABP (mmHg)	89.8 ± 4.7	95.4 ± 2.3	p > 0.317
Systolic BP (mmHg)	123.8 ± 7.8	137.7 ± 2.4	p > 0.05
Diastolic BP (mmHg)	67.8 ± 3.9	74.3 ± 3.5	p > 0.1
Bili (mmol/L)	15.7 ± 2.9	9 ± 1.46	p < 0.001
ALb (g/L)	41.2 ± 2.0	(n=4) 40 ± 1.1	p > 0.5
ALT (u/L)	53.3 ± 17.0	27.2 ± 5.12	p > 0.1
GGT (u/L)	314.7 ± 84.8	24.2 ± 6.0	p < 0.001
PTR ratio	1.03 ± 0.07	-	
Childs-Pugh Class	5A; 1B (mean pts 5.5 ± 0.3)	-	
Aetiology	4 ALC : 2 PBC	-	

Table 15

Subject characteristics for the forearm blood flow study (± SEM).

MATERIALS AND METHODS

The method was an open, single blind trial using plethysmography to assess forearm blood flow and its qualitative and quantitative response to local infusion of N-acetylcysteine in 6 patients with cirrhosis and 6 age matched controls without cirrhosis. In the absence of any previous studies of this type with N-acetylcysteine it was not possible to perform power calculations to define the number of subjects required for this study.

The patients/controls

6 with biopsy proven cirrhosis (5 Child's-Pugh A; 1 B) due to either alcohol or primary biliary cirrhosis, with proven portal hypertension i.e raised hepatic venous pressure gradient, ascites or endoscopically proven varices were recruited from an outpatient clinic (Table 15 and Appendix XV).

6 age matched controls without liver disease were found amongst healthy volunteers (Table 15 and Appendix XVI). As vascular response was studied, smoking status was also matched between patient and control groups (Appendix XV and XVI).

Exclusion criteria for all those taking part in the study included:

- Current hepatitis B,C or D
- Known cardiovascular disease
- Hypertension
- Bleeding diathesis or PTR more than 2.5:1 ratio
- Current vasoactive medication
- Pregnant women

Full written informed consent was obtained from each volunteer and patient and ethical approval for the study was obtained from Lothian Research Ethics Subcommittee.

Doses of N-acetylcysteine used for the study

N-acetylcysteine has not previously been administered intra-arterially to animals or humans and thus the rate of infusions (doses) used for this study i.e. 1mg/min, 3mg/min, 10mg/min, 30mg/min, 100mg/min and 300mg/min each administered for consecutive 10 minute periods were chosen to include a dose 1/100th of the systemic standard dose infused (50mg/Kg/min) in paracetamol poisoned patients/hepatic failure patients (Prescott et al, 1979 and 1989; Harrison et al, 1991). Approximately one hundredth of the systemic dose was chosen so that local rather than systemic effects of the drug could be studied. The N-acetylcysteine (20% w/v, Duncan Flockhart) was diluted in 0.9% saline for infusion.

The forearm blood flow response to incremental infusions of N-acetylcysteine was studied i.e. to determine a cumulative dose/response curve for the drug.

Study protocol

Each subject rested supine in bed with only one pillow under the head in a quiet warm, temperature controlled room (25-27°C) throughout each study period.

The forearm blood flow in both arms was measured by indium-gallium in silastic strain gauges arranged within a blood pressure cuff (venous occlusion plethysmography). The technique of plethysmography was first described by

Whitney in 1953. The technique involves inflation of wrist cuffs to 200 mmHg for periods of 3 minutes and intermittent inflation of upper arm cuffs to 40 mmHg over the same period (for 10s in every 15s cycle). The inflation was provided by a Junair air compressor. The subject's blood pressure was checked using a Takeda UA-751 automated blood pressure cuff.

A 10-20 minute equilibration period was used at the start of the study. The study only proceeded if the blood flow in both arms was similar and consistent.

A small volume of local anaesthetic (0.5 ml of 1% Xylocaine) was placed in the skin over the brachial artery of the non-dominant arm and a fine (27SWG) needle was then placed into the artery and a 0.9% (w/v) saline infusion started at 60 ml/hour, through epidural catheter tubing. The blood flow in both arms was then checked approximately 3 times over 30 minutes to allow full equilibration to take place.

Calibration recordings of 0.1% and 1% were made for each patient before small incremental doses of N-acetylcysteine were infused (doses are shown above) intra-arterially, each dose being infused for 10 minutes. All doses were infused at a rate of 60 ml/hour except the final dose of 300 mg/min which had to be infused at 90ml/hour because the maximum concentration of N-acetylcysteine available for human use was 200mg/ml (Parvolex, Duncan Flockhart, UK).

Blood flow in both forearms was recorded at 2-5 minutes and 7-10 minutes from the start of each new dose of NAC being infused. The blood pressure was checked between 5 and 7 minutes after the start of each new dose infusion.

Forearm blood flow recordings were made on a Macintosh microcomputer linked to a MacLab system.

Special precautions taken to achieve accurate recordings from the venous occlusion plethysmograph

The venous occlusion plethysmograph is an extremely sensitive instrument which is prone to artefact if special precautions in its use are not taken. Below is a list of precautions taken with each subject to improve reproducibility of recordings:

- 1 Before each study the volunteer was asked to empty their bladder. Thus there was no "stress" effect of needing to micturate and more importantly, once in place, the strain gauges did not change position.
- 2 Tight upper body clothing was exchanged for a light t-shirt and watches, bracelets etc were removed.
- 3 Subjects were told that once the equipment is in place they must not move either arm until the study was finished.
- 4 Foam pads were placed under each elbow, and doubled pillows were placed under each hand for support. The arms were at each side, semi-prone, with the hands 6-12" from the hips.
- 5 The thin inflation cuffs wound around each wrist were fixed with a 6" strip of "sleek tape".

- 6 The thicker inflation cuffs were wound around each upper arm, midway between elbow and axilla, as proximal as comfortably possible and fixed with an 8" strip of "sleek tape". They were just loose enough so that 2 fingers could be slipped between the cuff and the upper arm.
- 7 Each strain gauge was placed 6cm distal to the olecranon process. The strain gauge was moderately but not tightly stretched and the two wires were not crossed or kinked.

Calculation and statistical analysis of data

Forearm blood flow was expressed as ml per minute per 100 ml forearm as described by Whitney (1953). The blood flow in both the control and infused arm of the subject was calculated for each dosage. The overall forearm blood flow response to each drug was measured as the area under the dose-response curve (Matthews et al, 1990).

The ratio of forearm blood flow (infused arm/control arm) measured in response to N-acetylcysteine was expressed as a percentage of the ratio (infused arm/control arm) measured during the control period for each subject (Greenfield and Patterson, 1954).

All results are expressed as means \pm SEM and were compared using Student's t-test for paired or unpaired observations as appropriate, where $p < 0.05$ was considered statistically significant.

RESULTS

There was no significant difference in age, blood pressure, serum albumin or alanine aminotransferase activity between the control and cirrhotic subjects, as might be anticipated with Childs' grade A patients (Table 15). There were marked differences in serum bilirubin and gammaglutamyl transferase (GGT) activity between the two groups which reflects the aetiology of cirrhosis.

Baseline blood flows compared between the control arms of patients with cirrhosis and control arms of control subjects were not significantly different ($p > 0.317$) (Figures 39 and 40; Appendices XVII-XVIII). Also baseline blood flows compared between the infused arms of the two study groups were not significantly different ($p > 0.317$) (figures 39 and 40; Appendices XVII-XVIII). However, if the mean baseline flow in both arms of all patients in the cirrhotic group is compared with the mean baseline blood flow in both arms of the control groups, there is a slight statistical difference ($p > 0.046$) between the two groups (mean for both arms of controls = 3.87 ± 0.5 SEM; mean for both arms of cirrhotic patients = 3.01 ± 0.2). This is perhaps surprising and one might expect the cirrhotic groups to exhibit greater resting flow, however there are not at advanced stages of the Childs'-Pugh grading system.

However, the main function of this study was to establish if there was CHANGE in blood flow in the forearm in response to NAC in either groups of subjects.

Individual results of infused and control arm blood flows, infused/control forearm ratios and percentage change in forearm flow from baseline in response to NAC results are shown in Appendices XVII-XX. N-acetylcysteine caused a clearly seen large dose-dependent increase in resting forearm blood flow in all subjects (Figures 39 and 40 and Appendices XVII and XVIII).

As expected with infusion of a drug locally no significant changes were seen in mean blood pressure in either the control group or patients with cirrhosis from the baseline measurement to any time point during infusion of N-acetylcysteine.

Figure 41 shows the forearm blood flow ratios in response to N-acetylcysteine infusion. Once again the increased forearm blood flow response in each group is seen; there was no significant difference in haemodynamic response to any dose of NAC between the cirrhotic population and controls (Appendix XIX). This picture is also borne out by analysis of percentage change in blood flow from the predose value in response to each dose of NAC (Figure 42 and Appendix XX).

Forearm blood flow in response to N-acetylcysteine - controls

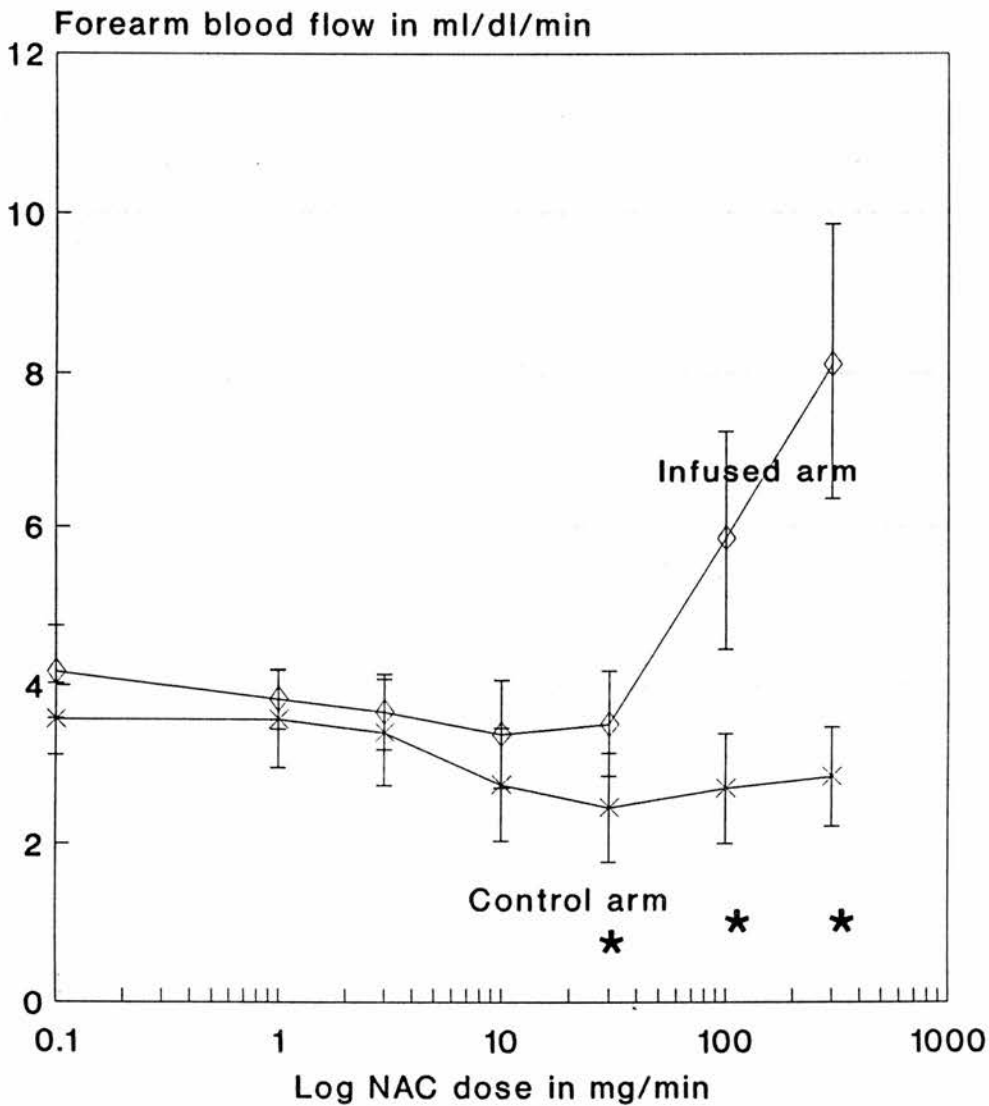


Figure 39

Forearm blood flow response to N-acetylcysteine - the control group.

* = significant difference between the control and infused arms at least to the 95% level.

Forearm blood flow in response to N-acetylcysteine - cirrhotics

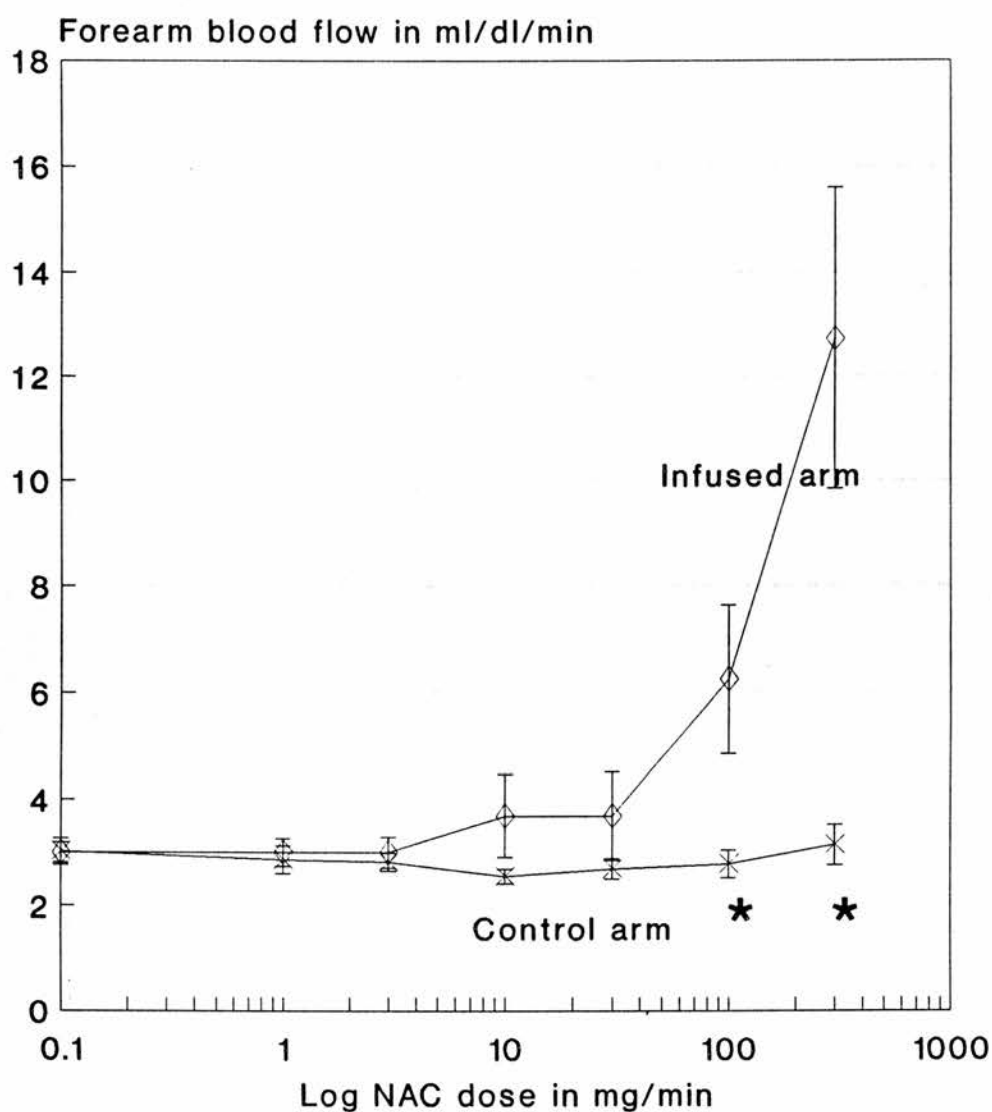


Figure 40

Forearm blood flow response to N-acetylcysteine - the patients with cirrhosis.

* = significant difference between the control and infused arms at least to the 95% level.

Forearm blood flow ratios in response to N-acetylcysteine

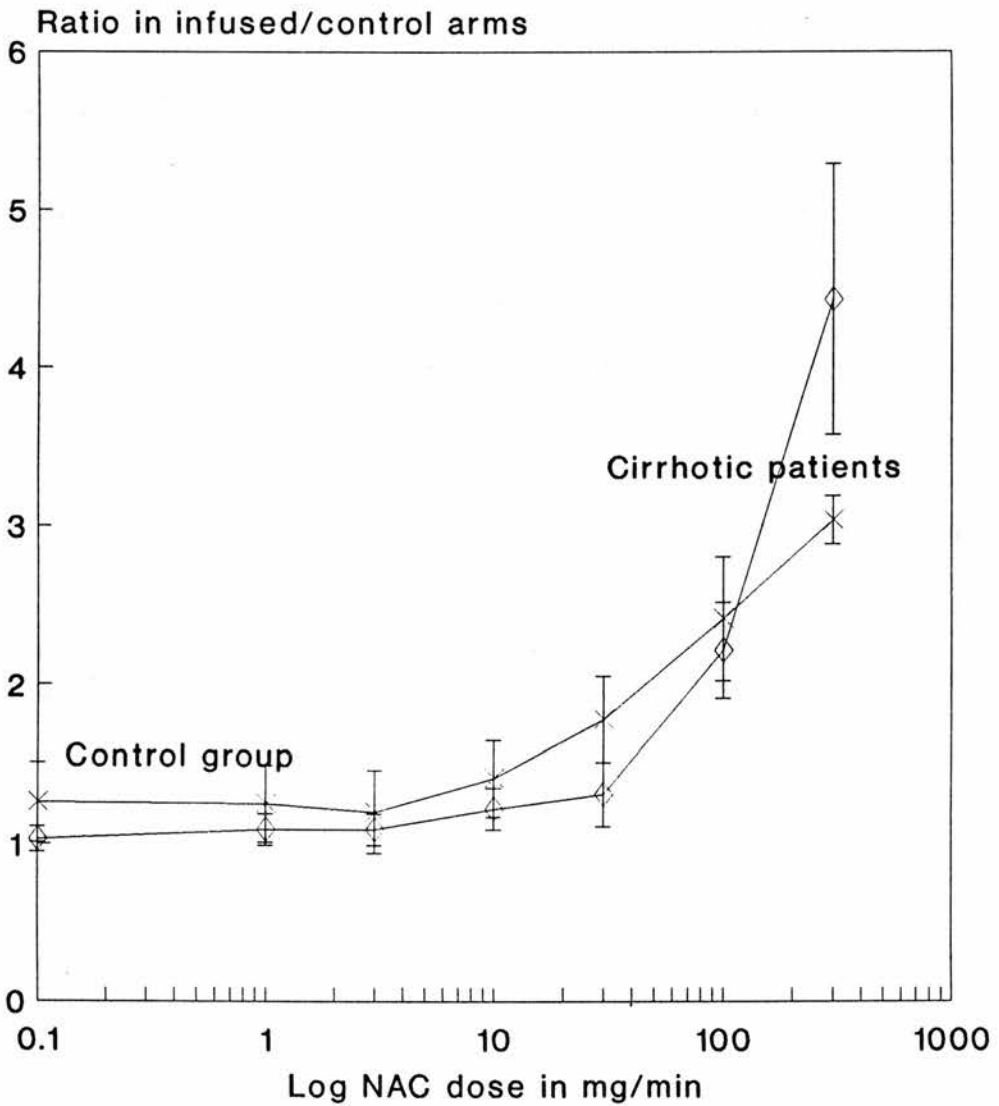


Figure 41

Forearm blood flow ratio (infused/control arm) in response to N-acetylcysteine - controls and patients with cirrhosis.

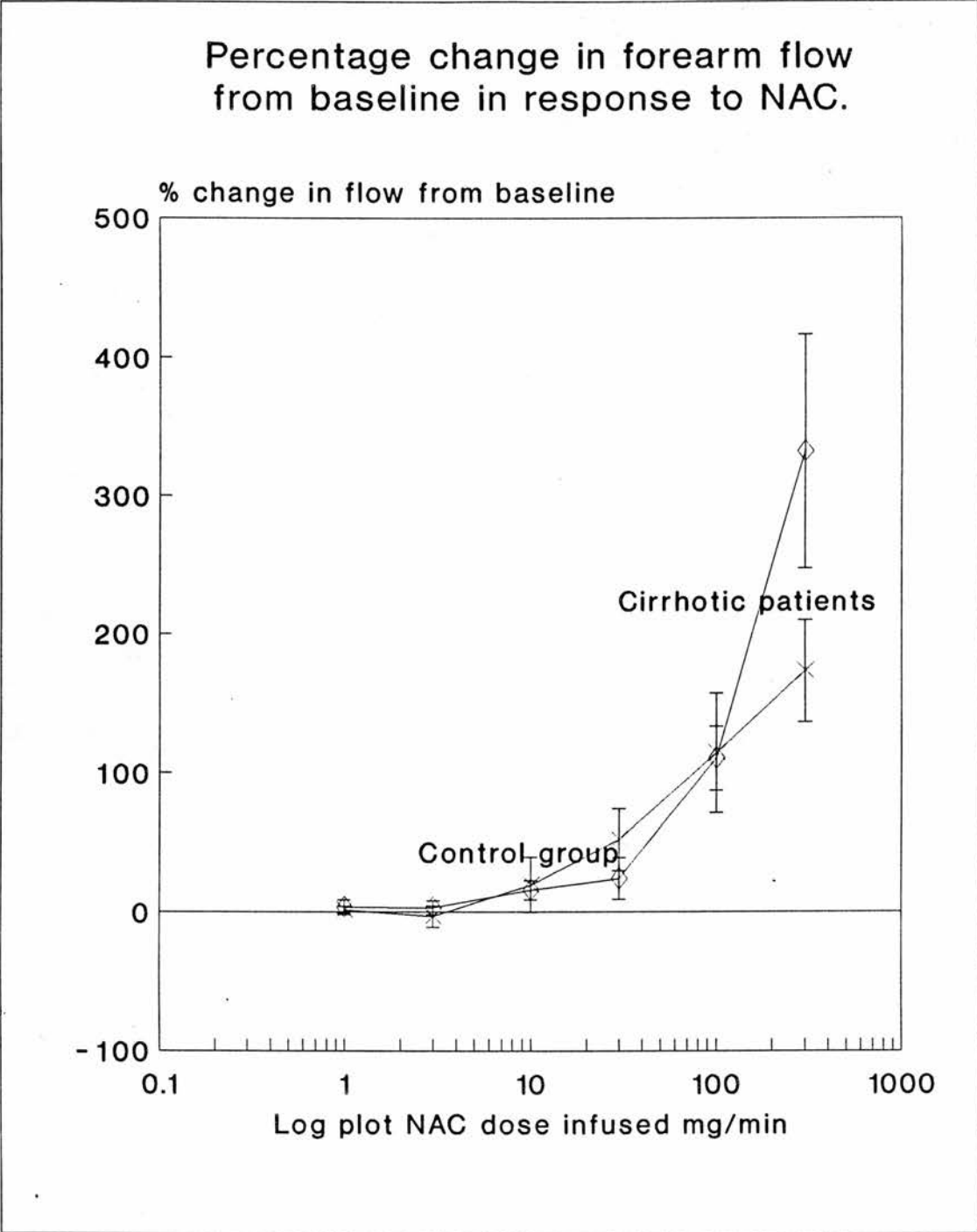


Figure 42

Percentage change in forearm flow from baseline in response to N-acetylcysteine - controls and patients with cirrhosis.

PRELIMINARY DISCUSSION

Measurement of the systemic haemodynamic effect of administration of drugs, such as described in Section III, does not distinguish between a local effect of the drug on the vasculature and central or reflex changes. However this study demonstrates that administration of NAC solution had a direct local vasodilation action, both in controls and in patients with chronic liver disease (Figures 39 and 40). From these dose response curves it appears to be a potent vasodilator.

Thus the action of NAC in Section III (ie reduction in systemic vascular resistance) is presumed to be due, in part at least, to a local vasodilation action. We cannot however extrapolate backwards from this and assume that the systemic haemodynamic effect of NAC in cirrhotics and controls will be the same as there are many other factors at play including vascular reflexes and inotropic action; this is discussed further in Section VI.

It is not clear to what extent the increased cardiac index after N-acetylcysteine infusion found in patients with cirrhosis (Section III) may be due to vasodilation but the rise in cardiac index in association with left ventricular stroke work index was suggestive of a positive inotropic response rather than purely vasodilation and reduction in left ventricular afterload.

The potential mediators for the vasodilatory effect of NAC are legion and will be discussed further in Section VI. The vasodilatory response to N-acetylcysteine was similar in patients with cirrhosis and healthy control

subjects. This implies that patients with cirrhosis, although they are known to be deplete in sulphurated amino-acids (Martensson et al, 1992), do not appear to be more sensitive to the effects of sulphydryl repletion by NAC than controls.

Criticisms of this study are that the numbers were small and that patients with more severe liver disease could not be included (due to logistical problems in transporting more unwell patients from the Royal Infirmary across the city to the Clinical Research Centre at the Western General Hospital). We know that the degree of haemodynamic disturbance and haemodynamic response to drugs in patients with cirrhosis may be related to the severity of liver disease (Valla et al, 1984; Braillon et al, 1986; Bendtsen et al, 1990). However, even in these patients studied with relatively mild cirrhosis, haemodynamic derangements were present prior to the study as manifest by a lower blood pressure (Table 15).

Unavoidable factors include baseline variability in haemodynamic measurements which may prevent the detection of differences in response between the controls and patients with cirrhosis, although as discussed in the materials and methods section, every effort was made to minimise these and the degree of variability (or greater) seen in our study was similar to other published forearm blood flow studies (Vallance et al, Lancet 1989; Calver et al, 1994). The method has been evaluated and found to be both sensitive and reproducible.

Several previous studies have however showed no difference in the haemodynamic action of drugs in patients with chronic liver disease, compared with healthy volunteers. For example, patients with cirrhosis and a control group given L-N-monomethyl-arginine (L-NMMA) both showed the same vasoconstrictor response to the drug and this argues against significant induction of NO synthase in the forearm arterioles of patients with mild-moderate cirrhosis (Calver et al, 1994). Interestingly the cirrhotic population in this study also tended to have mild cirrhosis; there were no Child's grade C patients in the study (Calver et al, 1994). The results of this study (Calver et al, 1994) were in contrast with experiments in rats with carbon-tetrachloride induced cirrhosis (Claria et al, 1992) and point to the importance of in vivo human studies, although undoubtedly they are hard to perform and may have many confounding variables, as discussed in detail in Section VI. The forearm blood flow response to local brachial artery infusion of noradrenaline in Calver's study (1994) did not differ between patients with cirrhosis and controls either, and suggests that the direct, local vascular response to a known potent vasoconstrictor is not diminished in patients with chronic liver disease.

Blood flow in the hands is predominantly through skin vessels and contains a high proportion of venous shunts, whereas forearm flow is predominantly through skeletal muscle. This makes comparisons of forearm blood flow studies and previous hand vein studies very difficult indeed and for this reason hand

vein studies are not discussed further; no hand vein studies reporting the action of N-acetylcysteine have been described in the literature.

This study demonstrated that patients with mild cirrhosis had the same (not significantly different) basal forearm blood flow as healthy age matched controls. One might expect the patients with cirrhosis to have increased blood flow when compared with controls (Murray et al, 1958; Calver et al, 1994). However the patients studied here had mild cirrhosis, almost exclusively Child's grade A, and we know that greater haemodynamic changes may be observed in those with more severe liver disease.

This study however, set out to achieve what was intended, to ascertain if NAC infusion caused local vasodilation in patients with cirrhosis - in this respect the data is unequivocal.

Section V

The pharmacokinetics of N-acetylcysteine in patients with cirrhosis

INTRODUCTION

For more than 25 years N-acetylcysteine (NAC), an analogue of cysteine, has been in clinical use. It has been used as a mucolytic agent (Scheffner, 1983) and is an effective antidote in paracetamol poisoning (Prescott et al, 1979). NAC is reported to offer protection against doxorubicin toxicity (Myers et al, 1983) and to reduce ifosfamide and cyclophosphamide induced cystitis (Botta et al, 1973; Holoye et al, 1983). NAC has also been used for reversal of the acquired tolerance to the cardiovascular effects of organic nitrates (Horowitz et al, 1988).

The biochemical features of N-acetylcysteine have been discussed in the introductory section (Section I). In Section III we showed that its effect on oxygen consumption is different in patients with cirrhosis from that reported by the Kings group (Harrison et al, 1991) in patients with hepatic failure or patients with cardiac disease.

Little is known about the metabolism of this important therapeutic agent and to date no-one has examined whether the presence of chronic liver disease alters its kinetics; of particular importance given its increasing use in patients with liver problems. It has however been suggested that in impaired liver function due to acute paracetamol poisoning the pharmacokinetics are not impaired (Prescott et al, 1989).

Better methods for the determination of NAC in plasma have appeared and thus the study of its pharmacokinetics is timely.

Therefore the aim of this study was to assess the primary and secondary pharmacokinetic parameters of NAC given intravenously to healthy man and compare these with patients with cirrhosis. Our hypothesis was that the pharmacokinetics may differ in patients with chronic liver disease as compared with normal controls.

MATERIALS AND METHODS

The population studied

The study group consisted of 9 patients with biopsy proven cirrhosis and six age and body weight matched healthy controls. Those with cirrhosis had their Bili, Albumin and ALT measured by standard laboratory methods in order to derive a Childs-Pugh score for each (Appendix XXI). Four of the 9 patients with chronic liver disease were concurrently receiving spironolactone. Patients on drugs other than spironolactone, and those with renal disease were excluded from the study. The 6 control subjects were recruited from the Department of Medicine staff: each drank less than 6 units of alcohol per week. No control subjects were taking any drugs or medication (Table 16). All subjects gave informed written consent and the study was approved by the local ethics committee.

Method of sample collection

The subjects did not take any other drugs or drink alcoholic beverages during the study. Following the method of Borgstrom (1986) the study was started at the same time of day for each subject who ate standardised food at a preset time.

A 21 G "butterfly needle" was placed in a vein in the antecubital fossa and secured with tape. A 5ml sample of venous blood was taken as a baseline. Then 3 ml of 200 mg/ml N-acetylcysteine solution (Parvolex, Duncan, Flockhart and Co) i.e. a bolus of 600 mg; 3676 μ mol was given intravenously to each subject in

	Control	Cirrhosis	p
Number	6	9	
M:F	4:2	7:2	
Mean weight (Kg)	72.5 (± 5.8)	67.3 (± 3.4)	NS
Mean age	39.8 (± 12.0)	51.1 (± 11.1)	NS
Age range	28-61	40-74	

(± SEM)

NS = no significant difference at the 95% level

Table 16

Baseline variables of the study population

the opposite arm by slow injection over 3 minutes (Borgstroem et al, 1986). This was "flushed" with 20 ml of normal saline.

Venous blood was sampled from the butterfly needle at 20 minutes, 40 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours, 8 hours and 10 hours after the NAC administration into plain blood tubes. On each occasion 2ml of venous blood was discarded from the tubing of the butterfly needle (roughly 2 ml) prior to sampling to avoid sample dilution with normal saline inside the tubing. After each sample the tubing was flushed with 10 ml 0.9% saline to avoid blockage of the tube.

Each blood sample was immediately centrifuged for 10 minutes at 3000 rpm in a Beckman bench centrifuge and the plasma was pipetted into a sample tube. The sample was stored at -40°C until quantitative analysis by HPLC (High performance liquid chromatography) was performed.

Sample preparation for HPLC

The method was a modification of that of Lewis et al (1984) and that used by Prescott et al (1989). Precision was improved by the addition of an internal standard (glycylglycine). Ultrafiltration of the reaction mixture as described by Lewis et al (1984) was found to be unnecessary and was omitted. The acidic derivative did not extract from the reaction mixture under the condition described by Lewis et al; and therefore in the method described by Prescott et al (1989) it was necessary to acidify the reaction mixture and re-extract it twice

with ether in order to achieve a satisfactory recovery of the derivative. The method measures **total NAC** i.e oxidised, reduced and protein bound drug.

Reagents for 20 determinations were prepared as follows:

- 1 Dithiothreitol 5g/L. 35 mg dithiothreitol was weighed and dissolved in 7.0 ml distilled water.
- 2 Stock glycylglycine 2g/L. 10 mg glycylglycine was weighed and dissolved in 5 ml of distilled water.
- 3 Glycylglycine working solution (0.1 g/L in 95% ethanol). 0.5 ml stock glycylglycine was diluted to 10 ml with ethanol.
- 4 Dinitrofluorobenzene (DNFB) solution (50 g/L). This was handled with care as it is a carcinogen. 220 mg dinitrofluorobenzene (recrystallised from ether) was weighed out and dissolved in 4.4ml ethanol.
- 5 Citrate/EDTA buffer. 29.41 g trisodium citrate dihydrate and 744 mg EDTA (disodium salt) were weighed and dissolved in 1500 ml distilled water; adjusted to pH 7.0 with 1 mol/L citric acid (422.03 g/200ml). This was then diluted to 2.0 L and filtered (0.45 μ m cellulose acetate).

Mobile phase:

methanol	30 }	mixed and purged with
citrate/ EDTA buffer	70 }	helium.

Subjects plasma (0.05-0.5mL) was placed in a 10 ml plastic tube; distilled water was added to a volume of 2 ml, followed by 0.3 mL dithiothreitol solution (5g/L). The mixture was mixed and incubated at 37°C for 30 minutes.

Sodium hydrogen carbonate solution (20 g/L, 0.7 ml) was then added, followed by 0.275 mL internal standard/reagent solution, prepared in advance by mixing 0.15 mL 2,4-dinitro-1-fluorobenzene, 5 mL ethanol and 1.73 mL glycylglycine (0.1 g/L in 95% ethanol) and pre-incubating 15 minutes at 60 °C. After incubating for 30 minutes at 60 °C, the mixture was cooled and centrifuged at 3000 rpm for 5 minutes.

The supernatant (1.0 mL) was transferred to a stoppered glass tube and washed with 5 ml ether by vortex mixing, centrifuging at 2500 rpm for 5 minutes and discarding the ether layer by suction at the water pump. Hydrochloric acid (1.0 mol/L, 0.5 mL) was added to the aqueous phase which was then extracted twice with ether as before: the ether extracts were combined and evaporated under nitrogen at 50 °C.

The dry residue was dissolved in 100 µl of mobile phase (citrate/EDTA buffer/methanol) and 10-15 µl was injected into the HPLC system.

HPLC (High performance liquid chromatography) for N-acetylcysteine

A Waters HPLC system was used using a U6K injector, M-45 pump and Model 441 fixed wavelength detector, 365 nm (sensitivity 0.2 aufs, chart speed 1 cm/min, pump speed 1 ml/min).

The column used was a 10 cm x 5 mm I.D. stainless-steel column (HETP) packed with Hypersil-ODS, 5 μ m (Shandon) by Capital HPLC specialists.

For each sample the peak height ratio of N-acetylcysteine to glycylglycine was measured (Appendix XXII). Glycylglycine and NAC eluted in 2.8 and 5.3 minutes respectively. The considerable help of Dr D R Jarvie, in this assay is acknowledged. A standard curve was plotted for calibration and used to read off the concentrations in the unknown samples.

Calibration standards

Stock solution (2g/L) was made by weighing 100 mg N-acetylcysteine (Sigma) and dissolving in 50 ml distilled water. The solution was used immediately.

Calibration standards:

mg/L NAC	10	20	40	60	80	100
2g/L stock solution	0.25	0.5	1.0	1.5	20.	2.5

All standards were diluted to 50 ml with drug-free serum (tested in the assay).

At the end of each batch of analysis, the column was washed and stored in 70 % methanol.

Within batch precision

10 replicates of freshly prepared 40 mg/L NAC in plasma.

Results (mg/L)	39.7	42.8	n = 10
	40.9	38.0	mean = 39.80
	41.3	41.3	SD = 1.89
	38.8	38.0	CV = 4.76%
	36.7	40.5	

Between batch precision

This was assessed by repeating assays of samples in the next HPLC run; values were always found to be within 2-5% of the previous estimations (approximately 20 assays).

The detection limit of the assay was 50 ug/L.

Pharmacokinetic and statistical analysis

The plasma concentration against time data for N-acetylcysteine were fitted to an open two compartment model by weighted non-linear regression analysis using the SIPHAR (Centre d'Etudes et de Recherches en Statistiques et Informatique Medicales, Cretail Cedex, France) pharmacokinetic curve fitting and modelling programme, Version 4.0 and the POWELL minimisation algorithm.

In addition to the coefficients of the model, this provided estimates of the area under the plasma concentration-time curve (AUC), total body clearance (dose/AUC), mean residence time (MRT) and the terminal plasma half-life of N-acetylcysteine for each subject.

RESULTS

Baseline variables for the patients with cirrhosis and healthy controls are shown in Table 16; from this it may be seen that there was no significant difference in age or weight between the two study groups. All the patients and healthy volunteers completed the study without adverse reactions.

A table of the estimations of plasma N-acetylcysteine at the time points for each subject are shown in Appendix XXIII and semilogarithmic plots of these are shown in Figures 43-45. In no individual (control or patients with cirrhosis) was endogenous NAC detectable in the baseline blood sample prior to administration of NAC.

Good fits of the N-acetylcysteine concentration versus time data to a two compartment model were obtained with all subjects using the POWELL algorithm. The pharmacokinetic variables for the patients with cirrhosis and the healthy controls are summarised in Tables 17 and 18 and each subjects individual pharmacokinetic analysis is shown in Appendix XXV. It can be seen that there is marked individual variation in pharmacokinetic parameters and this has also been found with other studies (Rodenstein et al, 1978; Borgstroem et al, 1986; Olsson et al, 1988; Burgunder et al, 1989; De Caro et al, 1989; Prescott et al, 1989).

Table 19 compares the pharmacokinetic parameters between the patients with cirrhosis and normal controls; from this it is clear that the volume of distribution at steady state is consistent with a distribution mainly in

Figure 43

Plasma NAC time-course - CONTROLS

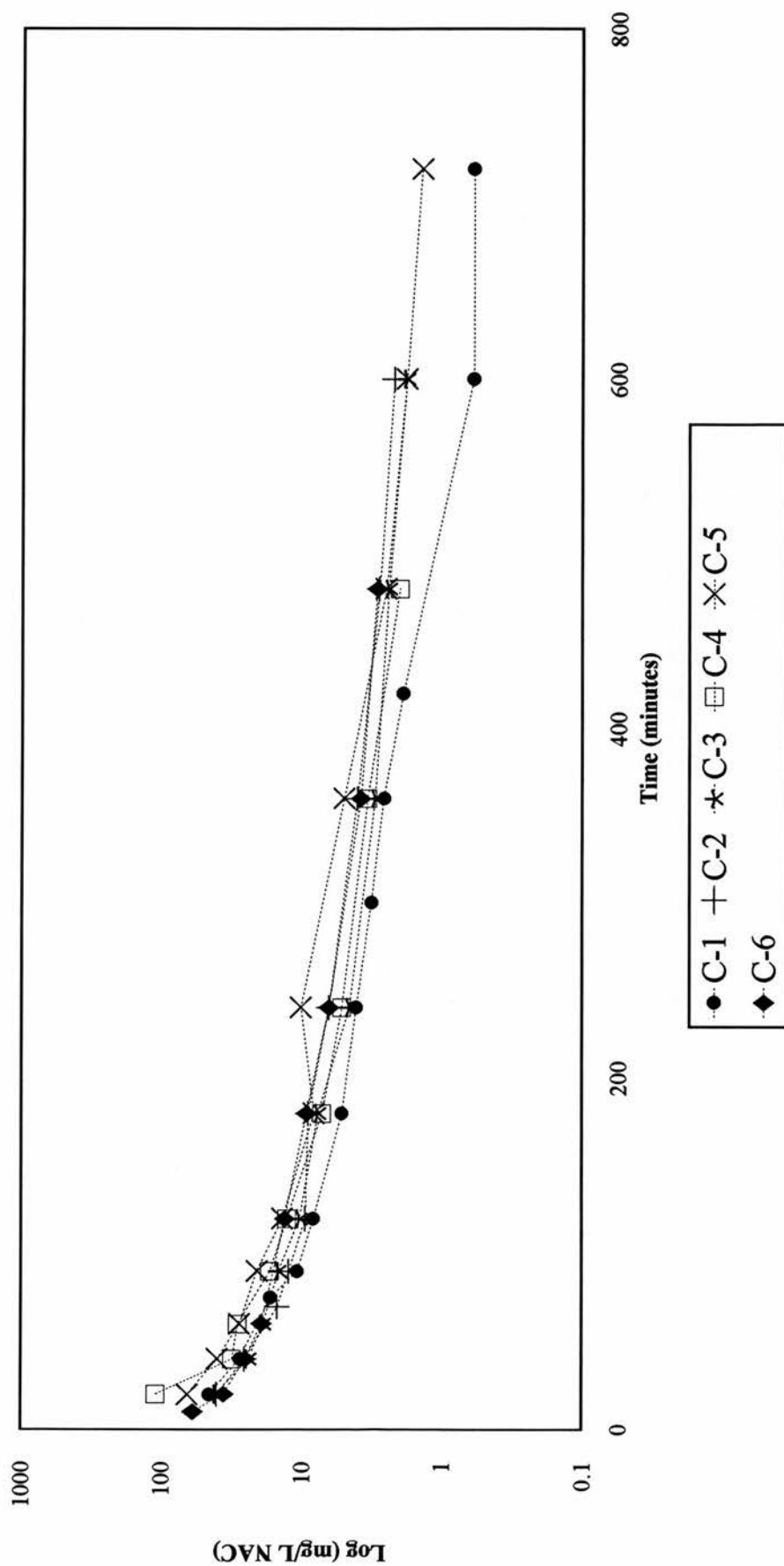


Figure 44 **Plasma NAC time-course - PATIENTS**

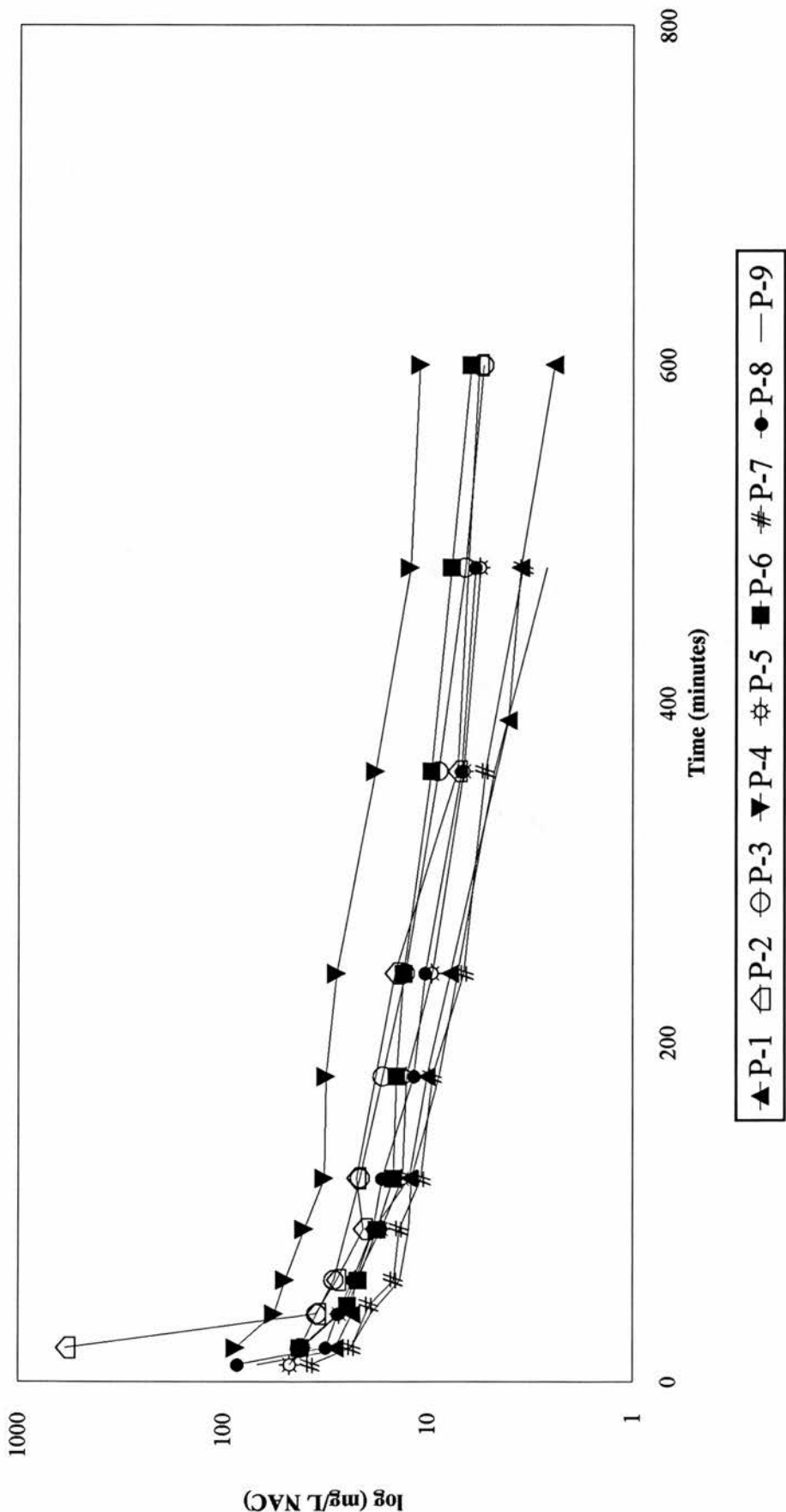
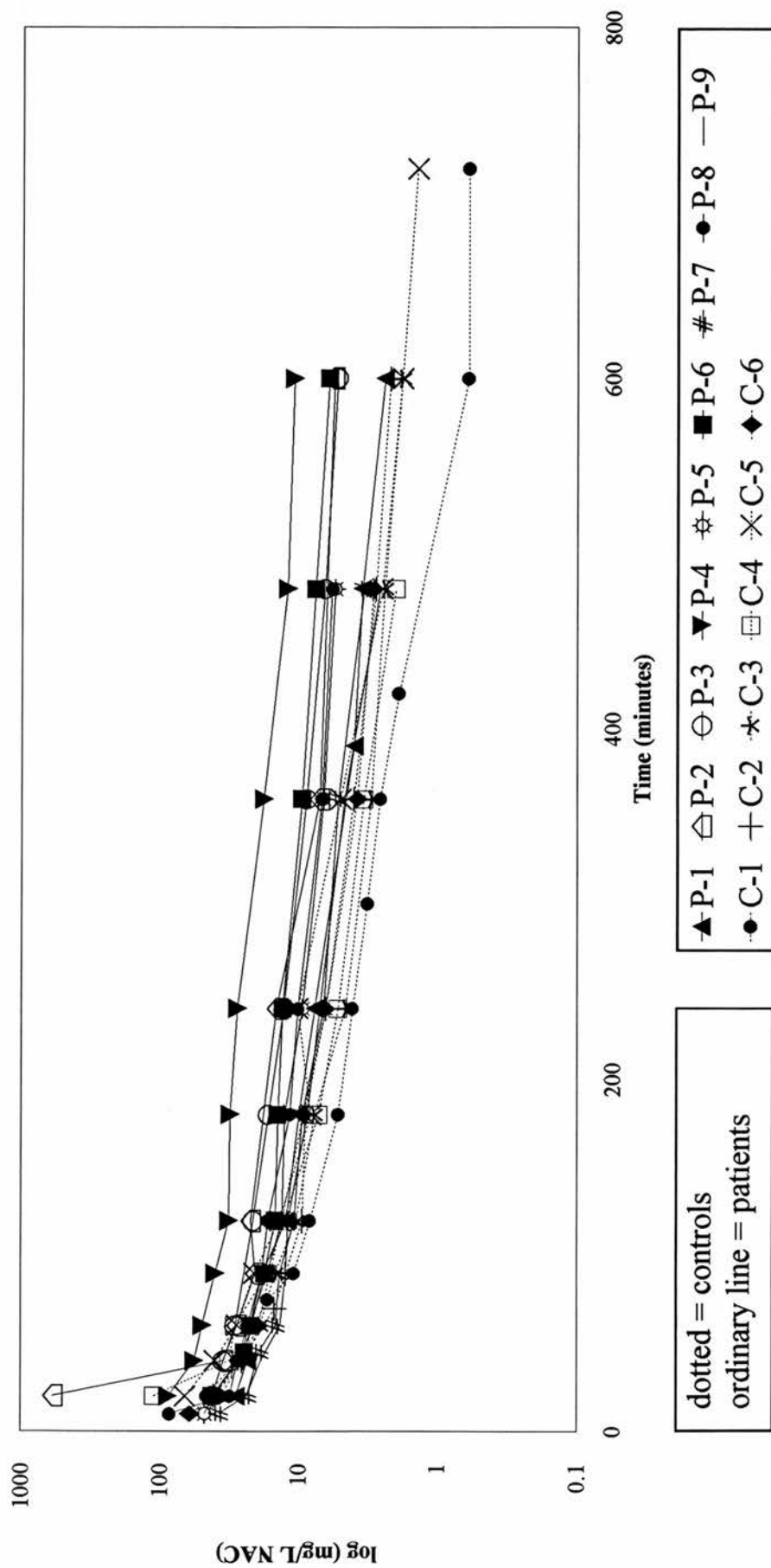


Figure 45

Plasma NAC time-course - PATIENTS & CONTROLS



NAC controls

Subject	t1/2 el (h)	AUC-exp (*)	Clr tot (***)	Vd (**)	MRT-exp (h)	Vdss-ex (**)	Vds-mod (**)
c-1	3.03	81.78	4.34	32.03	2.51	18.42	19.41
c-2	3.59	100.88	5.95	30.81	3.90	23.22	23.92
c-3	4.66	92.01	6.52	43.87	4.24	27.64	28.14
c-4	2.41	143.86	4.17	14.52	1.75	7.32	7.95
c-5	3.06	116.35	5.16	22.79	3.41	17.61	15.82
c-6	2.73	101.41	5.92	23.33	3.17	18.75	19.28
N	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Mean	3.25	106.05	5.84	27.89	3.16	18.83	19.09
SD	0.80	21.77	1.10	10.06	0.91	6.8	6.93
Min	2.41	81.78	4	14.52	1.75	7.32	7.95
Max	4.66	143.86	7	43.87	4.24	27.64	28.14

(*) mg /L x h
(**) mg / (mg/l) = litres
(***) mg / (mg/L x h) = litres/h

Table 17
Pharmacokinetic variables determined using the Powell algorithm for NAC in healthy controls.

NAC Liver disease patients

Subject	$t^{1/2}_{el}$ (h)	AUC-exp (*)	Clr tot (***)	Vd (**)	MRT-exp (h)	Vdss-ex (**)	Vds-mod (**)
P-1	5.08	107.86	5.56	40.78	5.22	29.06	29.52
P-2	2.79	385.20	1.56	6.26	1.40	2.18	6.04
P-3	5.91	190.40	3.15	26.87	6.83	21.52	21.71
P-4	4.48	350.52	1.71	11.06	5.87	10.05	10.16
P-5	4.53	140.93	4.26	27.83	5.62	23.93	24.05
P-6	5.87	189.87	3.16	26.78	7.51	23.75	24.30
P-7	3.47	93.92	6.39	31.98	4.46	28.48	28.61
P-8	3.32	191.61	3.13	14.99	3.33	10.44	12.36
P-9	2.80	126.05	4.76	19.25	2.49	11.83	15.06
N	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Mean	4.25	197.37	3.74	22.87	4.75	17.91	19.09
SD	1.22	103.60	1.64	10.90	2.02	9.50	8.44
Min	2.79	93.92	1.56	6.26	1.40	2.18	6.04
Max	5.91	385.20	6.39	40.78	7.51	29.06	29.52

(*) mg / L x h
(**) mg / (mg/l) = litres
(***) mg / (mg/L x h) = litres/h

Table 18

Pharmacokinetic variables determined using the Powell algorithm for NAC in patients with cirrhosis.

	t½ elim (h)	AUC (mg/L x h)	Vdss (L)	Clr tot (L/hour)
Controls (n = 6)	3.25 (0.80)	106.1 (21.8)	18.8 (6.8)	5.84 (1.10)
Patients (n = 9)	4.25 (1.22)	197.4 (103.6)	17.9 (9.5)	3.74 (1.64)
Unpaired t-test	p > 0.05	p < 0.05	p >> 0.5	p < 0.01

(± SD)

t½ elim = elimination half-life
AUC = area under the curve
Vdss = volume of distribution at steady state
Clr tot = clearance

Table 19

A comparison of the mean pharmacokinetic parameters of patients with cirrhosis and healthy controls following N-acetylcysteine administration.

extracellular water and the volume of distribution was the same in patients with cirrhosis and normal controls. With 4 cirrhotic patients showing significant ascites (Appendix XXI) it is a little surprising that the V_{dss} did not differ significantly between control and cirrhotic groups, however the standard deviation is marked.

The area under the curve was significantly different between the two groups and thus clearance was found to be impaired in cirrhosis compared with controls (Tables 17-19).

Correlations of the elimination half-life, clearance, volume of distribution at steady state and area under the curve were tested against serum albumin, bilirubin and PTR for the patients with cirrhosis using the Spearman Rank Correlation Test (Appendix XXV). No significant correlations were observed between any of the pharmacokinetic variables and Child's-Pugh variables in these patients.

PRELIMINARY DISCUSSION

Pharmacokinetics is the science of mathematical assessment of drug distribution in terms of model systems, of rate constants of transfer, of rate constants of metabolism and excretion, and of apparent volumes of distribution compartments.

The pharmacokinetics of N-acetylcysteine seen in our study patients and controls conform to a two compartment model. Single compartment models appear to be relatively rare in the distribution of foreign compounds such as drugs.

It will be noted that although Figures 43 and 44 are drawn with semilogarithmic axes they still have a concave shape; thus the first step in the analysis is the derivation of a suitable equation for each curve in Figures 43 and 44. With a two compartment model, it is necessary to postulate that the curve has two exponential functions:

$$C_{pt} = A \exp (-\alpha t) + B \exp (-\beta t),$$

where A and B are the intercepts at the y axis (concentrations at $t=0$) obtained from the semilogarithmic plot of C_p against t , and α and β are the rate constants of the two exponential components of the curve.

C_{pt} is the concentration of the drug in plasma and represents but does not equal the concentration in the body as a whole; in practice it is usual to measure the drug in plasma, not the whole body.

The constants in the above equation can be calculated by graphical methods based on trial and error or they can be determined using an appropriately programmed computer (such as shown in Appendix XXVI). It is possible to

introduce considerable degrees of error into later pharmacokinetic analysis by mistakes at this stage. The need to determine the most accurate equation from a collection of possibilities all of which appear to fit the data quite well cannot be overemphasised.

Having obtained a double exponential equation it is then appropriate to consider the details of the two compartment open system model (Figure 46). There are two compartments: the central compartment consists of plasma, blood cells and a group of rapidly perfused tissues including lung, brain, liver and spleen; and the peripheral compartment, consisting of a group of poorly perfused tissues, principally adipose tissue. Additionally there are at least two other compartments, of unabsorbed drug (dose) following oral administration and metabolised drug in urine and the gastrointestinal tract, awaiting final removal from the body, but these materials are not of importance in the present analysis.

Details of NAC distribution throughout the system were calculated from the data obtained from the graph of concentration against time. Thus:

$$\text{Apparent volume of the central compartment} = V_p =$$

$$\frac{\text{Dose}}{C_{po}}$$

$$\text{Rate of elimination} = K_{elim} =$$

$$\frac{A + B}{A/\alpha + B/\beta}$$

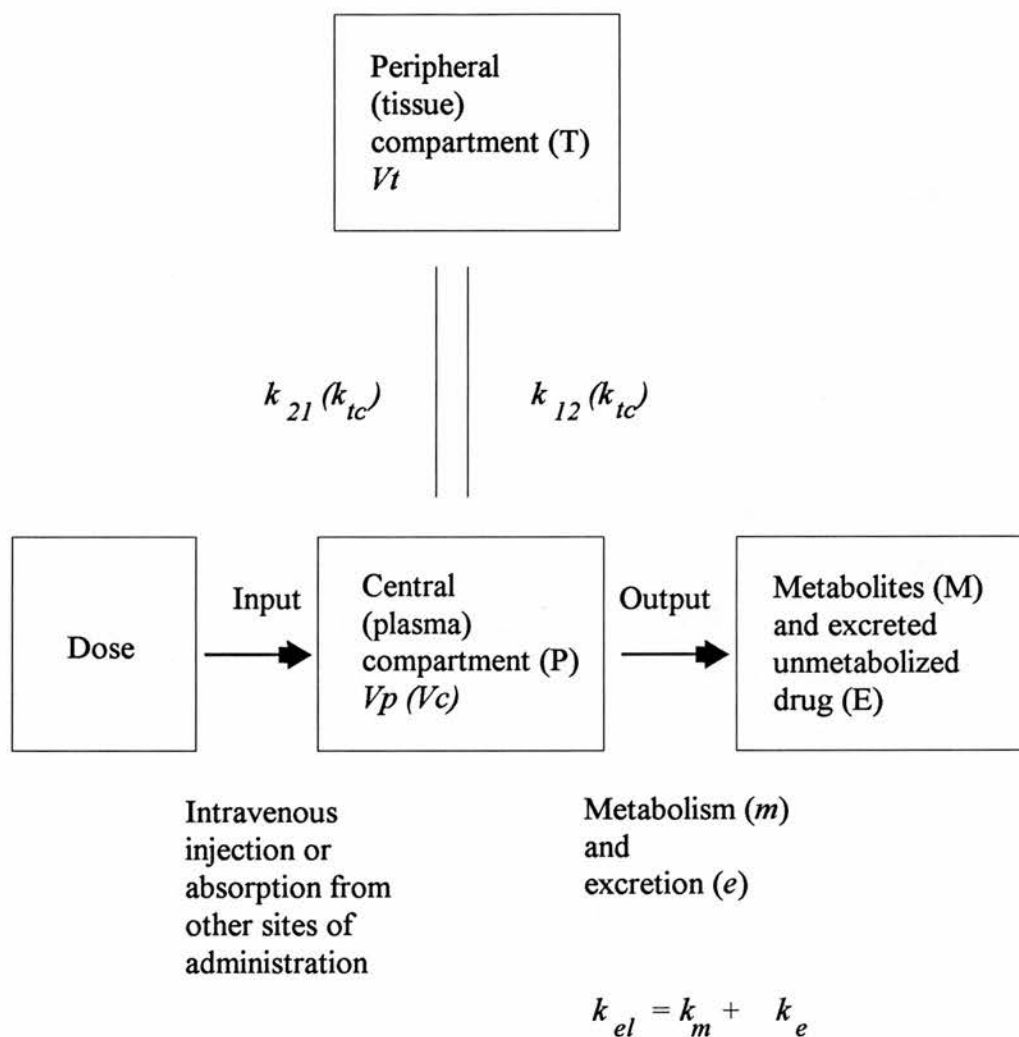


Figure 46

Two compartment model consisting of plasma, blood cells and a group of rapidly perfused tissues including lung, brain, liver and spleen.

Elimination half life (the time taken for the concentration to decline by 50%) = $t_{1/2 \text{ elim}}$ =

$$\frac{\ln 2}{K_{\text{elim}}} = \frac{0.693}{K_{\text{elim}}}$$

Area under the curve = AUC =

$$\frac{\text{dose given}}{V_{\text{dss}} \times K_{\text{elim}}}$$

Clearance = amount of drug distribution volume totally cleared of drug per unit of time = Clr_{tot} =

$$\frac{\text{dose given}}{\text{area under the curve}}$$

Rate constant of transfer from the peripheral compartment into the central compartment = K_{21} =

$$\frac{\alpha \times \beta}{K_{\text{elim}}}$$

Rate constant of transfer from the central compartment into the peripheral compartment = K_{12} =

$$\alpha + \beta - K_{21} - K_{\text{elim}}$$

Apparent volume of distribution at steady state = V_{dss}

$$= \frac{K_{12} + K_{21}}{K_{21}} \times V_{\text{p}}$$

These equations were derived in classical pharmacokinetic investigations. It is widely accepted that alpha and beta must differ by a factor of two if a pharmacokinetic analysis of this type is to be meaningful.

Few studies of the plasma concentrations and pharmacokinetics of NAC in man are available. Pharmacokinetic studies of NAC have been hampered by the lack of suitable analytical procedures to determine the compound in plasma. Previous studies have been performed with NAC labelled with ^{35}S (Rodenstein et al, 1978) and thin layer chromatography has been used for the separation and identification of NAC and its metabolites (Scheffner et al, 1966; Rodenstein et al, 1978; Bonanomi and Gazzaniga, 1980).

The results of previous studies of the kinetics of NAC in man have varied depending on the analytical method used, dose, formulation and route of administration (Prescott et al, 1989). For example, Borgstroem et al (1986) only assayed NAC in deproteinised plasma, no estimation of total NAC was thus made, whereas our study and those of Prescott and colleagues analysed total NAC i.e. including that bound to plasma proteins. By only measuring NAC in protein-free plasma, a relatively large fraction (often more than 50%) of the drug is lost, due to the fact that it is precipitated together with the proteins.

Total NAC comprises 3 fractions in plasma (Olsson et al, 1988; Burgunder et al, 1989):

- a free thiol
- b low-molecular weight disulphides
- c disulphides with SH-containing proteins

The three pools have been measured in the studies by Olsson and colleagues (1988) and Burgunder and colleagues (1989):

- a by omitting the incubation with dithiothreitol
- b by determining the difference in NAC concentration, measured in the presence of dithiothreitol, before and after removal of the proteins by ultrafiltration.
- c by subtracting a and b from total NAC.

No free thiol was observed in any sample; this is to be expected since disulphides form very rapidly during sample storage.

Borgstroem et al (1986) found their pharmacokinetic data best fitted a three compartment model, however our data and previous studies (Prescott et al, 1989) best fitted a two compartment model. This may reflect the fact that Borgstroem's group did not measure total NAC and the kinetics reflected by deproteinised samples may well be different.

Although we have estimated total NAC by the best method available to date the results however may not reflect its true disposition in vivo. In addition, NAC may in some cases act by releasing free cysteine though not in a 1:1 molar ratio

(Burgunder et al, 1989) and the concentration of the total drug may not therefore be a reliable index of its biological action. In addition NAC may exist as disulphides in vivo, or the HPLC method may not distinguish these from simple NAC.

The mean elimination half-life has varied from less than 2 to more than 6 hours in previous reports (Rodenstein et al, 1978; Borgstroem et al, 1986 [$t_{1/2} = 2.27$ hours]; Olsson et al, 1988 [$t_{1/2} = 6$ hours]; Burgunder et al, 1989, De Caro et al, 1989). This is consistent with the values found for our healthy controls and patients with cirrhosis i.e our control values compare well with previous small and full dose studies.

The values for clearance reported in the literature, for example 1.83 ml/min/Kg (equivalent to 7.68 litres per hour in a 70 Kg man: Olsson et al, 1988) and 3.5 ml/min/Kg (equivalent to 14.7 litres/hour in a 70 Kg man: Borgstroem et al, 1986) were of the same order but greater than the values seen in our study (Tables 17 & 18). Clearance was impaired in those subjects with cirrhosis.

The estimated volume of distribution in our study was 18.8 (+/- 6.8) for controls and 17.9 (+/- 9.5) for patients with cirrhosis, ie compatible with extracellular water. This would be expected as NAC is a sulphurated amino acid and is thus a hydrophilic compound and would be expected to distribute throughout extracellular water. The volume of distribution was of the same order as previous reports; Borgstroem et al (1986) report a value of 330 ml/Kg and Olsson et al, (1988) report a value of 470 ml/Kg. The disposition of NAC

does not appear to be dose-dependent (Prescott et al, 1989) or dependent of degree of liver disease (Appendix XXV).

NAC has been shown to have low bioavailability after oral administration (Borgstroem et al, 1986). This was assumed to be due to fast metabolism in the gut wall and liver. Our data showing that plasma concentration of NAC is higher in patients with liver impairment lends further substantiative evidence to this hypothesis.

Previous work has shown that NAC is stable when plasma is separated immediately after collection and stored at -20°C (Jarvie DR - personal communication). Furthermore there was no detectable loss of total NAC in plasma standards stored at -20°C for 6 months.

Prescott and colleagues used the modification of the method of Lewis et al (1984), measuring total NAC to show that NAC elimination was not impaired in patients with severe liver damage due to paracetamol overdose (Prescott et al, 1989) but we have shown that patients with chronic liver damage have higher levels of NAC than controls, with impaired clearance of the drug.

The disparity between these two studies may reflect the fact that maximum impairment of hepatic function is delayed for at least 3 days after overdose of paracetamol and it is possible that the enzymes/pathway involved in the metabolism of NAC were still functioning at this early stage or perhaps Prescott et al (1989) did not study severe enough patients to observe a difference. Other possibilities to explain the differences between this study and that of Prescott et

al (1989) includes greater utilisation of NAC by the liver in patients with paracetamol toxicity than those with cirrhosis, elimination from sites other than the liver, or that porta-systemic shunting is important in the cirrhotic group. The reduced clearance but lack of high C max suggests that porta-systemic shunting is not the major difference (which is just what you would expect from a low extraction drug).

On the other hand, the metabolism of paracetamol and some other drugs is impaired from the outset in patients who develop severe liver damage (Prescott et al, 1989).

NAC has been shown to be deacetylated in the liver in the rat (Sjodin et al, 1989), but it is also rapidly metabolized to cysteine and inorganic sulphite in the gastrointestinal tract. Other methods of analysis of NAC, clinical implications of the finding that the pharmacokinetics of NAC is altered in chronic liver disease, and suggested future studies are discussed in section VI.

Section VI

Discussion and conclusions

INTRODUCTION

Pharmacological agents are now widely used in the treatment of acute variceal bleeding, in the prophylaxis of the first haemorrhage and the prevention of rebleeding. Investigations continue on the mechanisms by which these drugs may be effective in portal hypertension.

THE ROLE OF HAEMODYNAMIC STUDIES IN THE ASSESSMENT OF DRUG ACTION

There is little doubt that in vitro studies of direct action of drugs on vasculature must be done but they do present significant limitations in the information that they can yield. A perusal of the discussion of the putative mechanisms of action of nitrate or N-acetylcysteine action (below) illustrates that in vitro work concerning drug action may be isolated to the system under study at best or even misleading to the in vivo situation.

Whilst direct measurement of for example, portal pressure, in animals provides little technical difficulties; the relevance to patients is of concern, particularly as animals have to be anaesthetised for measurements to be made and anaesthetic agents do have haemodynamic actions of their own. There are also the ethical issues in the use of animals for such studies and one must respect the majority public view in this regard.

In addition there is doubt whether for example the carbon tetrachloride exposed rat, provides a good model of the pathology of human cirrhosis; clearly

there are likely to be greater haemodynamic effects in the chronic alcoholic human than in the carbon tetrachloride exposed rat.

Thus, although of potential risk, human studies are of no doubt the greatest relevance to humans. As there appear to be so many variables in the haemodynamic action of drugs it is useful to be able to measure directly what action a drug has on various components of the haemodynamic system; particularly to differentiate between the portal and systemic haemodynamic effect (or indeed adverse effect). Thus the reason for the methods used in the studies here. For example, using such methods, adenosine receptor agonists have been shown to selectively act upon the portal system (Forrest, personal communication) and this presumably reflects the distribution of adenosine receptors within the portal system.

The aim of invasive haemodynamic studies is greater however than merely understanding the mechanism of action of drugs at different vascular beds. The aim is also to identify whom may benefit the most from drug action and who may be at risk from the haemodynamic effects; a correlation of benefit with a non-invasive parameter (such as a Child's-Pugh variable) is sought in an attempt to identify such individuals.

Haemodynamic studies may also be useful in identifying haemodynamic end-points for therapy such as a mean arterial blood pressure reduction to 70 mmHg. Bosch et al, 1993 have recently suggested that for the chronic treatment of portal hypertension, efficacy of therapy is greater when portal pressure is

reduced by 20% or more; though we feel that the drug's action on the collateral circulation may well be the most important variable.

CORRELATION BETWEEN CHANGES IN HEPATIC HAEMODYNAMICS AND OESOPHAGEAL VARICEAL PRESSURE

A recent study (Feu et al, 1993) correlated change in hepatic haemodynamics and oesophageal variceal pressure (measured with a non-invasive, pressure-sensitive endoscopic gauge) in cirrhotic patients either receiving propranolol or a placebo.

In eight patients the HVPG was unaltered or decreased by less than 10% after propranolol. In the remaining 13 patients treated with propranolol a reduction in HVPG was seen and this was associated with a significant decrease in measured variceal pressure and azygos blood flow.

However there was no significant difference between these 13 who did drop their HVPG in response to propranolol and the group of 8 "non-responders" with regard to the decrease in variceal pressure and azygos blood flow i.e. favourable effects are observed in some patients in whom HVPG fails to decrease by more than 10% of the baseline value and azygos blood flow does seem to be a good marker for a beneficial effect on oesophageal variceal pressure (Feu et al, 1993).

Standardisation of haemodynamic studies

During the studies described in Sections II, III and IV great care was taken to

standardise the invasive procedures as far as possible. Subjects were studied in a room of constant temperature, care was taken that the subjects were not actively withdrawing from alcohol or drinking at the time of the study.

Physical exercise during the studies was prevented as it may reduce hepatic perfusion (Iwao et al, 1993). Patients were standardised to the fasted state for the study; this was important as it has been shown that eating results in higher measured hepatic venous pressure gradients and precautionary in case operative intervention should be required if a complication of the procedure ensued.

Blood pressure recordings were made by staff in the haemodynamics laboratory who are well trained and aware of the difficulties of observer error. For each patient a single member of staff was responsible for measurement of blood pressure. In our experience a semiautomated device eg Takeda has been less reliable than a standard mercury syphygmomanometer as it tends to be "fussy" at crucial time points and thus distracts staff from making other vital concurrent recordings as they try to determine why it will not inflate the cuff, cannot find the systolic blood pressure etc. It could be argued that ideally, an intraarterial line should be used for assessment of mean arterial blood pressure but this is not without risk, particularly with patients with coagulopathy.

In Section III, the repeat haemodynamic measurement was made during the NAC infusion, thirty minutes after commencement of the infusion and the

infusion was continued for the further few minutes required to complete the haemodynamic measurements.

In Section IV the extensive precautions taken to standardise forearm blood flow recordings are listed.

THE HAEMODYNAMIC ACTION OF Is-5-Mn IN PATIENTS WITH CIRRHOSIS

Our study in Section II showed that both 10 mg and 40 mg of Is-5-Mn greatly reduced HVPG acutely and with chronic use and that there was no apparent advantage of one dose over the other in achieving HVPG reduction. A very important finding was the absence of pharmacological tolerance to isosorbide mononitrate in the patients studied, although a 16 hour nitrate-free interval was allowed each day.

The 40 mg dose appeared to induce a greater haemodynamic disturbance acutely i.e. increased heart rate, reduced mean arterial blood pressure and liver blood flow than the 10 mg dose; only the increase in heart rate effect was maintained after chronic use.

Only two previous groups showed no effect of nitrates on HVPG (Dawson et al, 1985; Tsai et al, 1989). All others showed a reduction in HVPG which was accompanied by a fall in cardiac index and mean arterial blood pressure.

As the efficacy of vasoactive drugs in either controlling an acute variceal bleed or preventing recurrent haemorrhage may be related to their ability to reduce blood flow through collateral vessels, determination of the effects of vasoactive agents on both collateral blood flow and HVPG is important in evaluating therapeutic potential (Feu et al, 1993). Our results indicate that the effect of 10 mg or 40 mg Is-5-Mn on azygos blood flow was highly variable including some

individuals in whom flow increased suggesting vasodilation of portasystemic collaterals. This finding has been indirectly confirmed by Forrest et al (personal communication) who have shown that flow through TIPSS may increase or decrease in response to Is-5-Mn and by Grose et al (1994) who found in some patients that azygos blood flow increased and in others it decreased in response to nitrates. In section II those patients who had low azygos flow increased their flow in response to nitrate and vice-versa. Thus the degree of pre-existing porta-systemic shunting appears important and this unfortunately suggests that there is unlikely to be a non-invasive way of predicting haemodynamic response to nitrates.

Severity of liver disease per se does not appear an important variable in defining haemodynamic response to nitrates as so few haemodynamic changes correlated with Child's-Pugh variables. A few correlations were positive but this would be expected by chance alone given the large number of correlations tested.

Other variables that have previously been suggested to be responsible for different response to nitrates between individuals may be degree of autonomic impairment (Moreau et al, 1989) or baseline cardiac filling pressure (Rector et al, 1990) but these variables were not assessed in this study as we felt that two invasive catheters were enough to manipulate quickly to allow valid correlative haemodynamic measurements to be made at each time point, particularly with the need for sampling from the hepatic catheter to allow estimation of liver blood flow by indocyanine green dye. As passage of a catheter through the heart

is associated with significant risk of arrhythmias we sought to minimise this, particularly as this was a chronic study and we felt this information had been assessed in previous studies.

A detailed comparison of the haemodynamic response to either dose of Is-5-Mn in our study to the previous literature (Tables 5-9 inclusive: Section I) is now made. Our study was designed to answer specific questions (Section II) and thus is different in experimental design to previous studies, in particular the assessment of effect of rechallenge with Is-5-Mn after one month. Despite these differences in experimental design it is noticeable that similar patterns of results emerge in comparison with the previous studies.

The slight (but not significant) increase in heart rate to 10mg Is-5-Mn over 1 hour (Figure 7) compares well with the effect of 5mg sublingual Isdo over 10 minutes (Hallemans et al, 1983), 5mg sublingual Isdo over 30 minutes (Merkel et al, 1987), 10mg oral Isdo over 1 hour (Blei et al, 1987) and 20 mg oral Is-5-Mn over 1 hour (Tsai et al, 1989; Navasa et al, 1989). Mols et al (1989) and Bhatia et al (1990) demonstrated a significantly increased heart rate in response to 5mg sublingual Isdo over 15-20 minutes. Also Hayes et al (1988) demonstrated a significant increase in heart rate to 20 mg Is-5-Mn over 60 minutes. Figure 9 shows that no significant heart rate difference from baseline was present at 1 month, even on rechallenge with 10mg Is-5-Mn. This compares well with studies using Isdo (Cervinka et al, 1989; Ikegami et al, 1992; Vorobioff et al, 1992) and Is-5-Mn (Tsai et al, 1989).

The acute heart rate response to 40mg Is-5-Mn (Figure 10) was greater than that seen with some smaller doses of Isdo in the literature as expected (Hallemans et al, 1983; Merkel et al, 1987; Blei et al, 1987) although Mols et al (1989) and Bhatia et al (1990) demonstrated similar increases in heart rate with 5mg sublingual Isdo. Interestingly, Blei et al (1987) did not demonstrate a significant increase in heart rate with 40mg Isdo and Navasa et al (1989) with 40mg Is-5-Mn over 1 hour which might have been expected from our results. A variety of reasons may explain heterogeneity of response to nitrates, as discussed above and in Section II discussion. Figure 11 showed a marked increase in heart rate after 1 month of 40mg Is-5-Mn bd ie there was no evidence of tolerance to the drug at 1 month. Cervinka et al (1989) however did not find a significant increase in heart rate after 80mg/day Isdo (slow release formulation) for 14 days, Ikegami et al (1992) after 40mg Isdo per day for 4 weeks or Vorobioff et al (1992) after 82 ± 10 mg/day for 65 ± 23 days. Similarly Tsai et al (1989) using 20mg Is-5-Mn bd for 1 week and Garcia-Pagan et al (1990a) using 40mg Is-5-Mn bd for 3 months failed to observe an increase in heart rate at 1 month. Several factors could account for this disparity including heterogeneity of response to nitrates, failure to provide an adequate "nitrate free interval" in previous studies and compliance with medication.

Figure 12 shows mean arterial blood pressure fell significantly in response to the acute administration of 10mg Is-5-Mn. This compares favourably with all the acute Isdo studies (Hallemans et al (1983), Dawson et al (1985), Merkel et al (1987), Blei et al (1987), Mols et al (1989), Bhatia et al (1990) and indeed with

previous Is-5-Mn studies (using higher doses of IS-5-Mn) (Hayes et al 1988; Tsai et al, 1989; Navasa et al, 1989).

Figure 14 shows mean arterial blood pressure fell significantly in response to acute administration of 40mg Is-5-Mn; as explained above this compares favourably with the previous literature.

Figures 13 and 15 show no sign of reduced mean arterial blood pressure after 1 month of either 10 or 40mg Is-5-Mn bd. This is similar to results observed with Isdo by Cervinka et al (1989), Vorobioff et al (1992) (although Ikegami et al, 1992 found a significant reduction of mean arterial blood pressure after 40mg/day Isdo for 4 weeks) and with Is-5-Mn by Tsai et al, 1989 (although Garcia-Pagan et al, 1990a found a significant reduction in mean arterial blood pressure after 40mg Is-5-Mn bd for 3 months).

Figures 16 and 19 show that WHVP fell significantly at all time points (except 10mg Is-5-Mn at $t=0$, 1 month) in response to acute and chronic administration of either dose of Is-5-Mn. This occurs and is of the same order as the results found with acute and chronic Isdo administration (Freeman et al 1985; Merkel et al, 1987; Blei et al, 1987, Qureshi et al, 1988; Mols et al, 1989; Cervinka et al, 1989; Ikegami et al, 1992; Vorobioff et al, 1992) and acute Is-5-Mn administration (Hayes et al, 1988; Navasa et al, 1989). Although Tsai et al (1989) and Garcia-Pagan (1990a) reported a fall in mean WHVP after chronic Is-5-Mn administration, this failed to reach statistical significance. The number

of patients studied by Tsai et al was small ($n=6$) and the Garcia-Pagan study was over 3 months which may account for the differences observed.

Figures 18 and 21 show reduced HVPG at all time points from the baseline level in response to either dose of Is-5-Mn, ie there was no evidence of tolerance. This compares well with Isdo (Hallemans et al, 1983; Freeman et al, 1985; Merkel et al, 1987; Blei et al, 1987; Mols et al, 1989; Ikegami et al, 1992) and Is-5-Mn (Hayes et al, 1988; Navasa et al, 1989; Garcia-Pagan et al, 1990a). The remaining studies showed no significant difference in HVPG but none showed a significant increase after Isdo/Is-5-Mn administration. The reduction of HVPG seen in previous studies is very variable and presumably reflects different doses of nitrate, severities of liver disease, porto-collateral shunting and cardiac filling pressures.

Unfortunately few studies in the literature have made measurements of azygos vein blood flow but, unlike other haemodynamic variables, marked differences in response to nitrates are seen in the two studies which have made such measurements (Navasa et al, 1989; Garcia-Pagan et al, 1990a). This is in agreement with the main message of the study in Section II i.e. that the pre-existing degree of portal-systemic shunting is important in determining response to nitrates. In Figure 26, Section II the term "arbitrary" indicates that the azygos flow groups have no precedent. However, they do have rationale as we have been aware whilst performing haemodynamic studies that patients with low flow may respond differently to pharmacological agents than those with high flow. The range of flows experienced in the haemodynamics laboratory was entirely arbitrarily divided into three groups and therefore no statistical analysis

was attempted on this data. Figure 26 is merely a way of visually representing an observation made in the haemodynamics laboratory that may well be of relevance in explaining the variability of response to nitrates in different patients. This is obviously an important idea on which to base a hypothesis for further studies as this information was not previously available.

Figures 27 and 28 show that there was no significant change in estimated liver blood flow after either dose of Is-5-Mn. This is similar to reports with Isdo (Merkel et al, 1987; Mols et al, 1989; Merkel et al, 1990; Ikegami et al, 1992) and Is-5-Mn (Navasa et al, 1989, 40mg dose only) and suggest that the fall in HVPg is due largely to a reduction in hepatic resistance rather than reduced liver blood flow. Clearly a reduction in liver blood flow would be undesirable.

However Hayes et al (1988) demonstrated a significant reduction of estimated mean liver blood flow from 1940 to 1639 l/hour after 20mg Is-5-Mn and Navasa et al (1989) demonstrated a significant increase from 890 to 1003 ml/min with the same dose. Garcia-Pagan et al (1990a) demonstrated a significant increase in liver blood flow from 1320 to 1510 after 40mg bd Is-5-Mn for 3 months. It would therefore seem that overall little mean change in liver blood flow can be anticipated in response to Is-5-Mn but individual differences may occur.

Comparison of Section II results with studies published after our study was commenced

Our findings in the 40 mg Is-5-Mn group have recently been confirmed by Silva et al (1993). Hepatic and systemic haemodynamic parameters were measured in

nine patients in basal conditions, after 1 hour and after 30 days of 40mg Is-5-Mn bd therapy. Rechallenge with nitrate does not appear to have been made on the second visit to the haemodynamics laboratory, unlike in our study. HVPG decreased from 15.1 ± 3.7 mmHg to 12.1 ± 5 mmHg at one hour and 11.3 ± 5.5 mmHg at 30 days, which was very similar to our figures. Estimated liver blood flow (ICG clearance method) was not modified thus estimated hepatic resistance decreased in both periods. The mean arterial blood pressure fell after 1 hour only - in keeping with our data. However, we showed that heart rate increased after 1 hour and remained significantly elevated at 1 month; data from Silva et al (1993) showed an initial increase that was not maintained. The fall in HVPG did not correlate with changes in portal or hepatic blood flow in our study or Silva et al (1993).

Grose et al (1994) studied the effects of acute and chronic administration of 20 mg bd Is-5-Mn on the portal and systemic circulation in patients with cirrhosis and portal hypertension. Acute administration (21 patients) reduced the MABP and HVPG (18.4 ± 0.9 to 16.5 ± 0.9 mmHg) whilst having a variable effect on azygos blood flow, as our data in Section II would suggest. Portal pressure fell consistently only in patients in whom the azygos blood flow increased acutely, which was not the case at the higher or lower doses used in our study (Grose et al, 1994). With chronic administration (8 patients), no reduction in MABP and HVPG was identified despite a marked and consistent reduction in azygos blood flow (540 ± 89 to 306 ± 60 mls/min). This seems a little surprising, given our results (Section II) and a review of the previous chronic

nitrate literature (Tables 7 and 8, Section I). However, Grose et al, 1994 showed that rechallenge with Is-5-Mn in patients on chronic nitrate therapy reproduced the haemodynamic effects identified with acute administration, lowering MABP and HVPG with a variable effect on azygos flow. The number used in the chronic study was small and the rechallenge effect raises the possibility of compliance as a factor.

However the differences between the study of Grose et al, 1994, the previous literature and the results in Section II probably simply reflect the variability in haemodynamic response that has been the characteristic of all the nitrate studies reported to date. The heterogeneity may also reflect a dose-dependent effect with different mechanisms of portal pressure reduction predominating at different doses; other factors such as cardiac filling pressures and autonomic effects may also add further to the variability seen.

COMPLIANCE AND SIDE-EFFECTS (NITRATES)

Our data suggest that the patients were taking their nitrate tablets during this study; their motivation to attend outpatient clinics was high and the discrepancy of tablet counts minimal. Side-effects were modest.

Adverse reactions associated with nitrates are unusual although mild headache resolving within 24 h of the first dose occurs in up to one-third of patients (Blei et al, 1987). In the first clinical trials of Is-5-Mn for prophylaxis of bleeding which are beginning to be reported, treatment did not have to be stopped because of side effects (Fassio et al, 1993).

THE MOLECULAR MECHANISM OF NITRATES

The molecular mechanism of vasodilation by nitrates is controversial. In 1977 two groups demonstrated a dose-dependent increase in the levels of cyclic-guanosine 5'-monophosphate (GMP) in smooth muscle after nitrate administration (Schultz et al, 1977; Katsuki et al, 1977).

Subsequently it was shown that many other vasodilators, including nitric oxide, activate soluble guanylate cyclase (Murad et al, 1978; Kukovetz et al, 1979).

Various possible mechanisms for the activation of soluble guanylate cyclase by the organic nitrates are discussed by Yeates (1992) but the mechanisms in intact blood vessels, in vivo and in blood vessel homogenates are probably different.

Nitrates may produce an increase in cyclic-GMP levels in smooth muscle by direct action or via nitric oxide production, and there is evidence for thiol intermediates as modulators of these cellular events (Horowitz et al, 1983 and 1988a) (Figure 47). Nitrates may directly stimulate nitric oxide synthetase to produce nitric oxide as demonstrated by the studies of Persson and colleagues (1994). They measured NO concentrations in exhaled air in anaesthetised rabbits and found that infusions of GTN induced dose-dependent increases in exhaled NO, which were abolished by L-NAME, a specific nitric oxide synthetase inhibitor. Similarly, the two metabolites of GTN, 1,2 and 1,3-glyceryl dinitrate were shown to produce a concentration increase in nitrite formation in an in vitro system, although the effects of NO synthetase inhibition were not studied (Salvemini et al, 1993).

NITRIC OXIDE (NO) SYNTHETASE

Endothelium

Vascular muscle

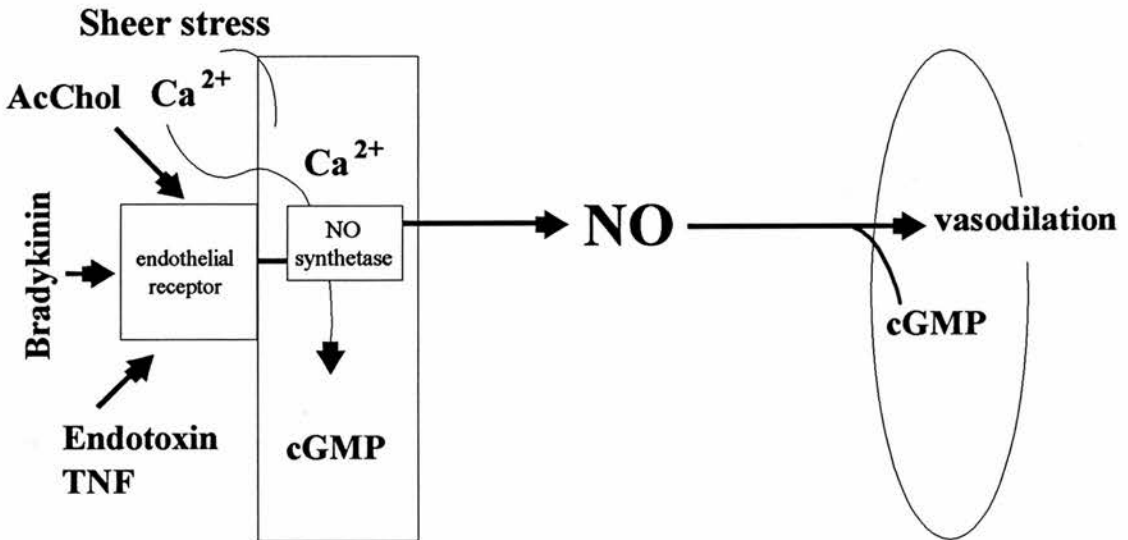


Figure 47

Nitric oxide as a vasodilator

Binding of vasodilators to endothelial receptors or shear stress activate nitric oxide synthase via a calcium mediated pathway.

Nitric oxide is generated from L-arginine and diffuses into vascular smooth muscle to cause relaxation after activation of soluble guanylate cyclase and generation of cGMP.

However, some vasodilators have been shown to generate nitric oxide in a nonenzymic reaction with cysteine (Moncada et al, 1988).

Nitric oxide may however not necessarily be the cause of the in vivo vasodilatory response to nitrates as illustrated by Vallance et al (1989) who showed that GTN infusion to normal volunteers caused a dose-dependent increase in forearm blood flow which was unaltered by L-NMMA, another NO synthetase inhibitor. N-acetylcysteine has also been shown to act as a vasodilator but its action may not be via nitric oxide production (see below).

MECHANISMS OF TOLERANCE TO NITRATES

Our study and others (Freeman et al, 1985; Cervinka et al, 1989; Ikegami et al, 1992; Vorobioff et al, 1992, Tsai et al, 1989; Garcia-Pagan et al, 1990a) showed tolerance to nitrates is not of concern in patients with chronic liver disease; several of these studies do not appear to have used a nitrate-free interval (Freeman et al, 1985; Cervinka et al, 1989; Vorobioff et al, 1992). This is a very important finding - previous chronic nitrate studies in patients with cirrhosis, and patients with ischaemic heart disease or cardiac failure have demonstrated tolerance to the haemodynamic effects of nitrate occur within a few days of commencing nitrate tablets. Thus follow up after one month was considered a reasonable time period for our study.

Multiple subcellular mechanisms of nitrate tolerance are postulated including conversion of the "nitrate receptor" to the disulphide form with lower affinity for nitrate (Fung et al, 1989), reduction of sulphydryl groups necessary for the "metabolic activation" of nitrates (Ignarro et al, 1981), reduction of vascular

production of nitric oxide (Chung and Fung, 1993), reduction in vascular metabolism of nitrate (Slack et al, 1989) or molecular alteration of intracellular guanylate cyclase (Waldman et al, 1986). These mechanisms although attractive for explaining in vitro phenomena, have been found inappropriate or inadequate for the in vivo situation. For example nanomolar concentrations of nitrates cause tolerance in patients versus millimolar concentrations in vitro on blood vessels (Fung, 1983) and tolerance occurs after several hours in patients yet develops under an hour in vitro. It is also difficult to see how these cellular biochemical mechanisms can be used to explain well known clinical phenomena such as nitrate resistance (Abrams, 1991) and withdrawal rebound (Olivari et al, 1983).

In addition, although sulphydryl donors can partially reverse GTN-induced tolerance in patients, this is not sufficient to implicate intracellular sulphydryl depletion as a mechanism of clinical nitrate tolerance (Fung et al, 1992).

However, a recent report suggests that higher than normal concentrations of N-acetylcysteine are found in the urine of patients with chronic liver disease and it would be interesting to speculate (Martensson et al, 1992) that endogenous N-acetylcysteine might also be a mechanism for less tolerance to nitrates in such patients, although we have assayed a 12 patients plasma for NAC (detection limit 50 micrograms/Litre) and failed to detect NAC (Dr David Jarvie - personal communication). If we had demonstrated tolerance to nitrates in patients with cirrhosis, it would have been interesting to see the effects of NAC administration.

Increased plasma renin, increased plasma catecholamines and body weight and sodium retention and shifts in vascular volumes have also been noted in those given nitrates for several days or more (Packer et al, 1987; Dupuis et al, 1990; Parker et al, 1991). These findings suggest that in vivo nitrate tolerance might be brought about by physiological compensatory mechanisms. Recent evidence suggests, as expected, that both subcellular and physiological mechanisms are involved in nitrate tolerance in vivo (Dupuis et al, 1990; Watanabe et al, 1993; Kertland et al, 1993).

A number of studies show that cirrhosis is accompanied by a variety of compensatory physiological effects eg. raised plasma renin (Bichet et al, 1982a), increased secretion of catecholamines (Henriksen et al, 1981; Nicholls et al, 1985) which may due to increased central sympathetic outflow (Floras et al, 1991) and alterations in vascular volumes (Henriksen et al, 1992). They therefore may not be able to develop further compensatory mechanisms of tolerance.

Strategies for the prevention of nitrate tolerance include the avoidance of maximum nitrate doses and the use of intermittent dosing regimens. Providing a relatively brief nitrate-free interval of 8-12 hours restores vascular responsiveness to nitrates, most likely due to recovery of the metabolic mechanism responsible for the therapeutic effect of these drugs (Amsterdam, 1992; Cowan, 1992).

THE RELATIVE SUSCEPTIBILITY OF ARTERIAL AND VENOUS SYSTEMS TO TOLERANCE TO NITRATES

There is still no general agreement concerning the relative susceptibility of the arterial and venous system to the development of nitrate tolerance in humans.

Zelis and Mason ,(1975), in a plethysmographic study, have shown that during a 6-8 week treatment with isosorbide dinitrate, cross-tolerance develops to the venous but not to the arterial dilator effects of nitroglycerin. Manyari et al (1985) found that during sustained therapy with isosorbide dinitrate, the effects of sublingual nitroglycerin on both venous volume and blood pressure were markedly diminished. The authors concluded that cross tolerance between isosorbide dinitrate and nitroglycerin develops in both the arterial and venous systems.

However, differing results were found in patients with congestive heart failure (Leier et al, 1983) who found that tolerance developed only to the systemic vascular effects without attenuation of venous and pulmonary vascular effects.

Makhoul et al (1990) however found in such patients that the acute nitroglycerin effects attenuated more rapidly and more extensively during a 24-hour infusion in the venous than in the arterial circulation. Ghio and colleagues (1992) demonstrated by measuring venous volume and forearm vascular resistance that after a 24-hour continuous infusion the effect of nitroglycerin was 50% reduced in the venous circulation in 20 patients with coronary artery disease but not in the arterial circulation. This is in keeping with the findings of Zelis and Mason (1975) and experimental data (Stewart et al,

1986) indicating that venous vessels are more readily susceptible to the development of nitrate tolerance than arterial vessels.

THE ROLE OF NITRATES IN THE PRIMARY AND SECONDARY PROPHYLAXIS OF VARICEAL BLEEDING

Preliminary clinical trials suggest a prophylactic benefit of nitrates for variceal bleeding (Angelico et al, 1993) although larger numbers of patients will be needed in future studies. Fassio et al (1993) studied 42 patients with cirrhosis and varices with "red signs" but whom had never bled. The percentage of patients free of bleeding 61 weeks after inclusion in the study was 62.4% in the group receiving Is-5-Mn and 43.6% in the placebo group. Such a clinical trial should examine a variety of doses of isosorbide-5-mononitrate as we have shown that 40 mg bd causes a sustained alteration in systemic haemodynamics and this may have long-term implications to patient wellbeing.

COMBINATION PHARMACOTHERAPY FOR ACHIEVEMENT OF HEPATIC VENOUS PRESSURE REDUCTION OR PROPHYLAXIS AGAINST VARICEAL BLEEDING

Although beta-blocking agents are accepted therapy for preventing first or subsequent bleeding episode, propranolol therapy decreases final HVPG to < 12 mmHg in only 12% of patients (Pereira et al 1991), while only 24% of patients have a > 20% reduction in HVPG and nearly 40% show no reduction in HVPG (Bosch et al 1984a, Garcia-Tsao et al 1986).

Therefore if we wish to increase the benefit of drugs to reduce portal pressure or azygos blood flow we must either select patients who exhibit a better haemodynamic response or develop combination therapies that can offer effective reductions in HVPG.

Table 20 shows drug combinations with proven or probable synergistic effects in reducing HVPG. Use of propranolol and isosorbide-5-mononitrate (Garcia-Pagan et al 1990b and 1991) achieves a greater reduction in HVPG than is possible with either drug alone. This may be due to isosorbide-5-mononitrate preventing the increase in portal resistance that is caused by propranolol (Bosch et al, 1993). Interestingly, the addition of isosorbide dinitrate to propranolol, although potentiating the reduction of HVPG seen with the latter, resulted in greater problems with management of ascites (Vorobioff et al, 1993). This however was not confirmed in a study of combination Is-5-Mn and propranolol (Morillas et al, 1994). The beneficial effect on azygos blood flow is maintained whilst liver perfusion and hepatic function appear to be unchanged with nitrates plus beta-blocker (Garcia-Pagan et al 1991; Morillas et al, 1994). Whether the enhanced haemodynamic effects of this combination therapy translate into better clinical results must be verified by randomised, controlled studies.

A new agent that combines the effects of beta-blockers with those of nitrates, nipradilol, was tested in portal hypertensive rats with synergistic action on HVPG reduction; clinical trials are awaited (Ohsuga et al, 1993).

Table 20 Drug combinations with probable synergistic effects in reducing portal pressure - clinical studies

Propranolol and nitrate	Haemodynamic response measured
Garcia-Pagan et al, 1990 (Propranolol: 0.1mg/kg, then 2mg/h iv. Is-5-Mn: 20 or 40mg (1 dose p.o.)	27% reduction HVPG
Garcia-Pagan et al, 1991 (Propranolol: dose increased until heart rate reduced 25% for 3 mths p.o. Is-5-Mn: 40mg bd for 3 mths p.o.)	19% reduction HVPG
Vorobioff et al, 1993 (Propranolol bd: dose increased until heart rate reduced 25%, mean dose 159 +/- 69mg/d: Isdo to avoid reductions in MABP of more than 20%, mean dose 40 +/- 17mg/day)	21.6% reduction in HVPG
Tincani et al, 1993 (40mg propranolol and 60mg sustained release Is-5-Mn on 2 consecutive days)	Duplex doppler sonography demonstrated reduced portal vein flow in more patients on combination therapy than either drug alone
Propranolol and ketanserin	
Hadengue et al, 1987 (Propranolol: 15mg, 1 dose iv: ketanserin, 5mg, 1 dose iv)	19% reduction HVPG
Spiro nolactone and propranolol	
Garcia-Pagan et al, 1991b (Spironolactone: 100mg/day for 2 months p.o.: propranolol 0.15mg/kg 1 dose iv)	25% reduction HVPG

Similarly, in another rat study, combination therapy with SMS 201-995 (a somatostatin analogue) and low dose Isdo may be more effective at reducing portal influx and portal pressure than either alone (Hori et al, 1994).

Whilst combination therapy is theoretically attractive the difficulty with compliance, particularly in patients with alcoholic liver disease, must be borne in mind.

Future research

Better methods are required to identify high risk patients appropriate for pharmacotherapy of portal hypertension. This is especially important for the selection of patients for treatment to prevent first variceal haemorrhage as 65-75% of these will not experience variceal bleeding. Further studies are necessary to evaluate the combination of pharmacological agents and sclerotherapy or endoscopic variceal ligation. Studies evaluating drug combinations to lower HVPG more aggressively are also needed.

RENAL EFFECTS OF DRUGS USED IN TREATMENT OF PORTAL HYPERTENSION

Drugs used to lower HVPG may have adverse effects on sodium and water metabolism and ascites formation. Propranolol has marked effects on systemic haemodynamics, the sympathetic nervous system and the renin-angiotensin system but does not appear to have much effect on renal function, including salt and water handling (Wilkinson et al, 1977; Bataille et al, 1984; Rector and Reynolds, 1984; Bernardi et al, 1989; Henriksen et al, 1992; Vorobioff et al,

1993). Circulating noradrenaline increases during treatment with propranolol, indicating enhancement of alpha-adrenergic tone (which increases salt and water retention), but the activation of the renin-angiotensin system decreases (which decreases salt and water retention), as reflected by reduced circulating levels of renin, angiotensin II and aldosterone. Thus the overall effect on the kidney is negligible in Child's A and B patients (Bataille et al, 1984). However, there is only limited experience of treatment with propranolol in advanced cirrhosis (Bendtsen et al, 1991).

Clonidine treatment over a prolonged period of time results in a reduction in arterial blood pressure which theoretically would be expected to have an adverse effect on renal function. This however was not found to be the case in one study (Albillos et al, 1992b).

Nitrates have recently been shown to reduce renal blood flow, glomerular filtration rate, free water clearance and renal sodium excretion (Salmeron et al, 1993). A single oral dose of nitrate produced a significant reduction of renal function and sodium and water excretion, an effect most likely caused by the observed reduction in MABP. Despite a comparable reduction in MABP between compensated and decompensated patients with cirrhosis, a more severe decrease in kidney function occurred in the decompensated patients, which may have been caused by greater activation of the sympathetic nervous system and renin-angiotensin-aldosterone axis and a shift in renal autoregulation (Henriksen and Ring-Larsen, 1993). It is interesting to note that in spite on the theoretical benefits of combination nitrate and beta-blocker

administration on renal side-effects in portal hypertension that the study of Vorobioff et al, (1993) demonstrated that the combination still impaired renal function significantly, probably due to arterial hypotensive effects.

Diuretics or ACE inhibitors have well known adverse effects on renal function (Daskapoulas et al, 1987a and b). Because the kidneys in cirrhotic patients behave like bilateral Goldblatt kidneys (Lassen et al, 1986), the fall in renal perfusion pressure may have a serious adverse effect. A marked hypotensive effect in patients with an activated renin-angiotensin-aldosterone system has been observed with ACE inhibitors in cirrhosis (Daskapoulas et al, 1987b). Although an increase in renal blood flow takes place because of a reduction in renal vascular resistance, the cause is apparently a decrease in tone of the efferent glomerular arteriole. The end-result therefore is a decrease in GFR, the filtration fraction and, finally the urine volume.

Calcium channel blocking agents cause a reduction in mean arterial blood pressure but renal effects have only been reported in one study (Lay et al, 1987).

Similarly serotonin S-2 receptor-blocking agents reduce mean arterial blood pressure but the question of impairment of renal function remains to be answered.

Two adverse effects of medical therapy may preclude drug used for portal pressure reduction; liver dysfunction and adverse renal effects. The predominant factor that appears to affect renal function is the reduction in

arterial blood pressure, as further reduction of the already characteristically low blood pressure will reduce renal blood flow and glomerular filtration rate, with subsequent reduction in sodium excretion and free water clearance. All this information must be taken in context that a significant % of patients are non-responders to portal hypotensive agents (especially beta-blockers) and therefore we are potentially placing some patients at risk of renal or liver dysfunction for little-no benefit.

THE HAEMODYNAMIC ACTION OF N-ACETYLCYSTEINE

i) NAC as a vasodilator and positive inotrope

Section IV has shown that NAC acts as a vasodilator in patients with cirrhosis and in normal controls from a rate of infusion of 30 mg/min into the forearm on the cumulative dose/response curve (Figures 39-42). There were small changes seen at an infusion rate of 10 mg/min. The response to NAC has been studied as a cumulative effect of each test dose being infused over 10 minutes. It is possible that the dose-response curve may look different if each dose were tested on separate occasions but this is clearly impractical with 6-8 doses.

The control group showed less vasodilation in response to N-acetylcysteine than the patient group, although this difference was not statistically significant.

However the patients had relatively mild cirrhosis (mainly Child's group A) and thus if a more severe group (and perhaps larger numbers) were studied a statistical difference from healthy volunteers response to NAC might be seen. This would be expected since patients with cirrhosis are known to be depleted in

sulphurated amino-acids (Martenssen, et al, 1992). N-acetylcysteine is a sulphhydryl donor and we know that SH donation is important in vasodilatory mechanisms from in vitro work on vascular rings; thus NAC might be expected to have a greater vasodilatory effect in cirrhosis.

Whether NAC possesses direct relaxant activity in normal vascular smooth muscle in vitro is controversial. Torresi (et al, 1985) and Munzel (et al, 1989) suggested it did not. The work of Munzel and colleagues however showed that it potentiated the vasodilatory response to nitroglycerin. A more recent study by Sunman et al (1993) however showed that NAC caused an endothelium-independent relaxation of rat and human resistance arteries. What is not always clear from some in vitro studies is the degree to which intact endothelium was present in the vessels tested, as absence of endothelium (and hence endothelins, nitric oxide etc) may well impede vascular response to a drug. In addition the size of the vessel may be important (Wheatley et al, 1994) and the length of time of NAC exposure (Boesgaard et al, 1994a).

Administration of NAC intra-arterially to rabbits at 20 mg/Kg produced a significantly lower peripheral vascular resistance but administration at 200 mg/Kg produced no significant change (Knight et al, 1990). Perhaps then some of the differences between in vivo studies reported in the literature result from different doses of NAC used. It may be that at high doses NAC has some toxic effect.

Although we have shown that normal controls have a local forearm vasodilatory response to N-acetylcysteine in vivo, this does not mean that systemic

haemodynamic features would necessarily occur in controls, as reflex changes which compensate for the vasodilation may occur. Prior to our studies there was little evidence to suggest that NAC had any haemodynamic action in healthy controls (as discussed below). However our forearm blood flow study suggests that given locally, at certain doses, NAC acts as a vasodilator both in healthy controls and patients with cirrhosis.

In Section III we showed that NAC given at a rate of 150 mg/kg for 15 minutes, followed by 50 mg/Kg for 15 minutes to patients with cirrhosis resulted in increased cardiac output and reduced systemic vascular resistance and was intended to be directly comparable with the work of Harrison (et al 1991) and Packer (et al, 1987).

As NAC administration to the 11 patients with cirrhosis resulted in raised cardiac index and peripheral vasodilation and as most drugs that primarily cause afterload reduction increase cardiac index without affecting left ventricular stroke work index, the rise in cardiac index in association with left ventricular stroke work index here is suggestive of a positive inotropic response rather than purely peripheral vasodilation and reduction in left ventricular afterload.

Positive inotropic action was also found in the study of Harrison (et al, 1991) and Walsh (unpublished observation). This action is unlikely to be due to hyperosmolality but may be due to a direct effect of the drug on the myocardium, eg due to -SH donation, free radical scavenging or nitric oxide

myocardium, eg due to -SH donation, free radical scavenging or nitric oxide production. This requires further study. It will be very important to determine if NAC's action as a positive inotrope is a transient effect or continues throughout a prolonged infusion period. If the effect were prolonged, this might explain the reduced need for inotropic agents in patients treated with NAC in fulminant hepatic failure (Keays et al, 1991). Similarly the time course of the peripheral vasodilation of NAC requires evaluation.

We cannot predict whether healthy volunteers would have shown the same responses to systemic infusion of NAC as cirrhosis, given this regime, but the literature to date suggests that NAC would have no haemodynamic action:

Administration to healthy and shocked pigs (Groeneveld et al, 1990; Modig and Sandin, 1988), patients with ischaemic heart disease (Horowitz et al, 1983; Winniford et al, 1986), patients with heart failure (packer et al, 1987) and patients who had recovered fully from fulminant hepatic failure (Harrison et al, 1991) produced no haemodynamic changes.

ii) NAC as an antidote to nitrate tolerance

NAC has been given intravenously (5g in 200 ml 5% dextrose over 15 minutes) to patients with coronary artery disease who had all received pretreatment with a nitroglycerin infusion, some of whom had developed tolerance in the arterial and venous beds to the nitroglycerin. In the patients with "venous tolerance" to NAC the venous volume increased significantly after N-acetylcysteine infusion

but in the remaining patients no change occurred (Ghio et al, 1992). No change in forearm blood flow was seen in response to NAC in these patients (previously given nitroglycerin) (Ghio et al, 1992). This is seemingly in contrast with previous studies indicating potentiation of nitroglycerin action with NAC.

Much of the discrepancies in the literature may be attributed to differences in the protocol of NAC administration. In this respect it would appear that pretreatment with NAC potentiates the effects of a subsequent infusion of nitroglycerin. The increase in coronary blood flow (Winniford et al, 1986) or the decrease in systemic blood pressure and pulmonary capillary wedge pressure (Horowitz et al, 1983) in response to a fixed dose of nitroglycerin was significantly higher after NAC than under control conditions.

However, in a similar study to Ghio et al (1992), where NAC was added to a previous 24-hour infusion of nitroglycerin (May et al, 1987), only reversal of nitrate tolerance without potentiating effect was observed; however these authors did not carry out a separate analysis in patients who developed and who did not develop nitrate tolerance.

To confound things further, only partial reversal of tolerance was reported by Packer et al (1987) when NAC was given in eight nitrate-tolerant patients with congestive cardiac failure. Also a non-specific potentiation of nitroglycerin action has been demonstrated (Munzel et al, 1989) and it has been postulated

to occur at an extracellular site (Fung et al, 1988). We are still unable to explain fully the conflicting results obtained in humans.

The incubation of tolerant arterial vascular rings with different sulphydryl donors produced reversal of tolerance in most (Needleman and Johnson, 1972; Torresi et al, 1985) but not all (Abdollah et al, 1987) studies. Clearly the interactions between organic nitrates and sulphydryl groups are so complex and incompletely elucidated that we cannot currently explain the diversity of response dependent on different patient groups, and when different doses and schedules of NAC are given in vivo. The putative subcellular mechanism of action of NAC are discussed below.

iii) The effect of NAC on O₂ delivery and consumption

Below a critical O₂ delivery, systemic O₂ consumption is maintained relatively constant by proportionate increases in tissue O₂ extraction (Russell and Phang, 1994), ie O₂ delivery decreases in the physiologically normal state.

Many clinical studies of acute respiratory distress syndrome, septic chock and critical illness purport to show dependence of O₂ consumption on O₂ delivery, even when O₂ is delivered in a supranormal range but such dependence is not replicated in animal models and may only appear to occur due to mathematical coupling of errors in the measurement of shared variables used to calculate O₂ delivery and consumption (Archie, 1981; Russell and Phang, 1994). Ronco (et al, 1993a) demonstrated that sepsis did not alter the critical O₂ delivery for anaerobic metabolism or tissue O₂ extraction ability. He also showed that in septic patients

having therapy withdrawn that the lower end of the delivery of O_2 /consumption of O_2 relationship was the same as in normal controls (Ronco et al, 1993b).

The Walsh study (personal communication) has demonstrated that O_2 consumption does not increase as O_2 delivery increases in fulminant hepatic failure patients given NAC regime, ie the same results as we found with cirrhotic patients. Why might the Harrison study have found differing results from the Walsh and our studies? As mentioned previously, the relationship between two haemodynamic variables may be mathematically coupled when they are assessed, for example by the Swan-Ganz catheter technique. If either one or both are derived and/or calculated this can lead to erroneous results (Archie, 1981). Thus an apparent difference may be seen which is not actually the case. It is possible to correct for such bias in a linear correlation coefficient by use of the first two terms of a Taylor Series expansion of the function (Stratton et al, 1987).

It is possible using the Swan-Ganz catheter technique to find a spurious increase in apparent O_2 consumption, because as the arterio-venous gradient narrows and the cardiac index rises, any sampling error is magnified enormously (Bartlett and Dechert, 1990).

In additions patients with cirrhosis have functional pulmonary shunting (Rodriguez-Roisin et al, 1992) and NAC may act as a vasodilator to increase pulmonary shunting. This hypothesis is supported by the observation that the pulmonary vascular resistance index fell in response to the NAC infusion and that the arterial pO_2 fell in certain patients.

The exact nature of such shunting is controversial (Berthelot et al, 1966) and may be physical or physiological. Recent evidence of normalisation of ventilation/perfusion relationships after liver transplantation in patients with decompensated cirrhosis indicates that these changes have a direct functional relationship to the diseased liver (Eriksson et al, 1990).

Physiological mechanisms contributing to the hypoxaemia of cirrhosis include a right shift in the oxygen dissociation curve which occurs in many patients (Rodriguez-Roisin et al, 1992) due to an increase in 2,3 diphosphoglycerate but this alone is insufficient to explain the degree of hypoxaemia.

The multiple inert gas elimination technique (Evans and Wagner 1977) has shown ventilation/perfusion (V/Q) mismatching may be responsible in some patients. Patients with lower pulmonary vascular resistance have greater V/Q mismatch (Rodriguez-Roisin et al, 1992). Increased ventilation/perfusion mismatch might be expected to be the most likely mechanism for the blood gas changes observed in our 11 cirrhotic patients in response to NAC infusion (Section III; Table 13).

A wide range of gas exchange abnormalities may occur in patients with chronic liver disease. Whether the mechanism underlying some of these is related to failure of metabolism or of production of one of several circulating vasoactive substances by the damaged liver cells or to altered metabolism of paracrine factors synthesized by endothelial cells is unknown (Rodriguez-Roisin et al, 1992). Many potential pulmonary vasodilators including prostacyclin, ANF and

PAF have been detected therefore potentially contributing to depression of hypoxic pulmonary vasoconstriction.

iv) Other reasons for differences between the action of NAC in cirrhosis and fulminant hepatic failure

Haemodynamic differences in response to NAC between these two groups of patients may also be due to the fact that some of the fulminant patients had encephalopathy (+/- neurohumoral factors) and were on mechanical ventilation (volume controlled) with continuous intravenous infusion of muscle relaxant (Atracurium 50 mg/hr). Pulmonary capillary wedge pressure had to be stabilised at 8-14 mmHg by infusion of 4.5% human albumin solution before the study.

v) NAC as a protective agent

Reduced glutathione (GSH) is the main intracellular low molecular weight thiol which acts as a nucleophilic scavenger and as an enzyme-catalyzed antioxidant in the event of electrophilic/oxidative tissue injury. Therefore, GSH has a major role as a protector of biological structures and functions, for example, GSH repletion by early administration of NAC protects the liver from paracetamol induced liver damage.

Lack of GSH and electrophilic/oxidative injury have been identified among the causes of the adult respiratory distress syndrome (ARDS), idiopathic pulmonary fibrosis (IPF) and AIDS. Experimental and early clinical data (in ARDS) point to the role of NAC in the treatment of these conditions. Recently oral NAC has

been shown to enhance the levels of GSH in the liver, in plasma and notably in bronchoalveolar lavage fluid. In healthy volunteers, the rise in glutathione following systemic NAC administration, was not due to increased liver production and splanchnic efflux (Poulsen et al, 1993). Rescue of GSH through NAC needs to be appreciated as an independent treatment modality for an array of different diseases, all of which have one feature in common; loss of GSH.

Although in Section III we have shown that NAC is not of haemodynamic benefit in cirrhosis, we would still advocate its use in paracetamol poisoned patients with cirrhosis; however it would seem prudent to monitor arterial oxygen tension and avoid the use of other positive inotropes if possible.

THE MECHANISM OF ACTION OF NAC AS A VASODILATOR/REVERSER OF NITRATE TOLERANCE

Sulphydryl groups appear to be required for the relaxation of vascular smooth muscle. In cirrhotic patients who are known to be deplete in sulphurated amino-acids (Chawla et al, 1984) NAC may act by sulphydryl repletion to cause vasodilation (Figure 48). Alternatively, NAC may be acting through production of nitric oxide. Endothelium derived relaxing factor (nitric oxide), synthesized by endothelial cells relaxes vascular smooth muscle (Palmer et al, 1988) and inhibits the aggregation and adhesion of platelets (Radomski et al, 1987); both actions are potentiated by NAC (Stamler et al, 1989).

NITRIC OXIDE (NO) SYNTHETASE

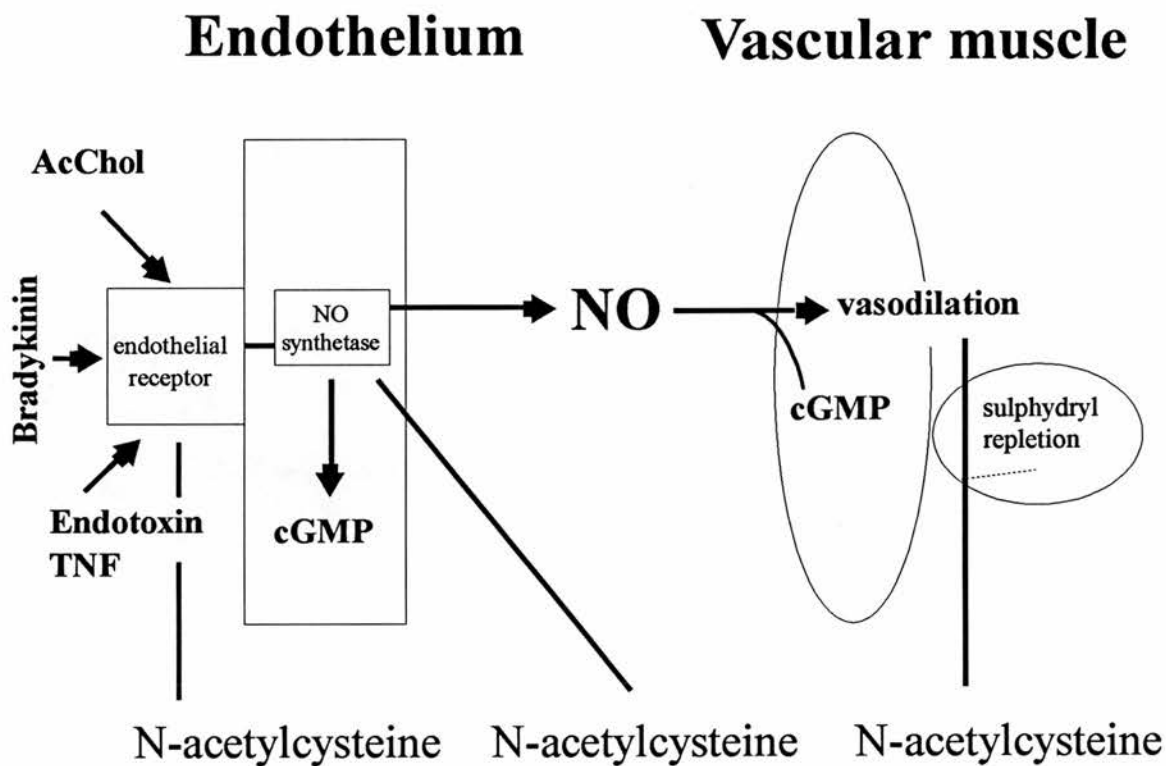


Figure 48

N-acetylcysteine - possible mechanisms of action as a vasodilator.

A recent study has shown that significantly enhanced production of nitric oxide from GTN in plasma was observed with a whole variety of sulphydryl compounds (Chong and Fung, 1991; Chung et al, 1993). Comparative in vitro relaxation studies were carried out using isolated (and nitrate tolerant) rat aortic rings with GTN/thiosalicylic acid (an aromatic thiol) or GTN/NAC, in the presence of 0.5% plasma. Under these conditions partial reversal of GTN tolerance could be achieved with TSA, but not with NAC (Chong et al, 1991). These data are consistent with the view that extracellular production of nitric oxide or S-nitrosothiol serves as a tolerance-reversing mechanism of thiols on GTN. TSA appears to be a more potent sulphydryl compound than NAC in this biochemical and pharmacological interaction.

Lahera and colleagues (1993) have shown that sulphydryl donors potentiate the hypotensive effect of acetylcholine in rats; an effect which is inhibited by L-NMMA. It was suggested that the mechanism was therefore nitric oxide-dependent but that nitric oxide was protected from free radical destruction by formation of a S-nitrosothiol compound with NAC.

NAC is unlikely to lead to release of nitric oxide by stimulation of TNF production however as a recent study from Milan (Peristeris et al, 1992) has shown that NAC inhibited TNF production and hepatotoxicity in mice treated with lipopolysaccharide.

A recent effect of thiol containing agents in reducing prostaglandin I₂ production via increased cGMP by cultured human vascular endothelial cells has been reported (Kobayashi et al, 1991).

Thus NAC may be acting directly on vasculature or indirectly via nitric oxide (most likely) by sulphydryl donation (Figure 48) (Myers et al, 1990). It is difficult to test the hypothesis that the mechanism of action of NAC involves nitric oxide using the forearm blood flow model (for example using L-NMMA) as there is no haemodynamic change to assess that could be attributed merely to the NAC working through nitric oxide i.e. there are too many confounding variables.

The effect of NAC on reversal of nitrate tolerance is stereospecific for the L-isomer which suggests it is enzyme/ receptor dependent for its mechanism of action (Newman et al, 1990). Initial claims that it failed to reverse tolerance to Isdo have recently been refuted and SH groups have been shown to be very important in reversal of tolerance (Mehra et al, 1994). However, a recent study suggests that haemodynamic tolerance to nitrates in major vessels is not due to depletion of vascular thiol compounds (ie cysteine and glutathione) necessary for the bioconversion of organic nitrates (Boesgaard et al, 1994b). Much about the role of thiol groups in tolerance and reversal of tolerance to nitrates remains to be elucidated.

Alternatively, like other vasodilators, NAC may activate endogenous neurohormonal systems. Boesgaard and colleagues (1993) demonstrated that

N-acetylcysteine inhibits angiotensin converting enzyme in vivo. Perhaps even both mechanisms occur but affect different vascular beds eg SH donation might be the dominant mechanism of action in peripheral veins whereas neurohormonal activation may be the mechanism of action in arteries. This concept, though appealing, ignores the possibility that these two mechanisms can strongly interact. Since nitrate tolerance has been explained by both direct loss of pharmacological effect due to reduced bioconversion and an indirect effect due to activation of the renin/angiotensin system and counterregulatory vasoconstriction, from the mechanisms of action described above, it becomes easy to see how NAC may ameliorate tolerance.

In addition it has been shown that NAC may also act as an antioxidant (Gressier et al, 1993), reductant and free radical scavenger or one of its metabolites notably L-cysteine, glutathione and sulphate may be the active molecular species (Sjodin et al, 1989). Another possible mechanism of action of NAC as a vasodilator and positive inotrope has been suggested by our finding that stock NAC solution (200mg/ml) has an osmolarity of 2400 mOsm/L (checked by osmometer), of which approximately 1200 mOsm is due to the sodium ion (standard biochemical assay). The sodium concentration is so high because NaOH solution is added to NAC to make the solution neutral for injection.

The osmolarity of the solutions of NAC infused in the forearm blood flow study (Section IV) were calculated as:

Osmolarity of solution
(mOsm/L)

100 mg/min NAC = 1.36 mOsm/min	1360
30 mg/min NAC = 0.67 mOsm/min	670
10 mg/min NAC = 0.43 mOsm/min	430
3mg/min NAC = 0.35 mOsm/min	350
1mg/min NAC = 0.32 mOsm/min	320

A review of the literature on the effect of hyperosmolar solutions suggests that this could be responsible for the vasodilatory action of NAC. Overbeck and Grega (1970), infused hypertonic sodium chloride solutions at a rate of 1,2 and 4 mOsm/min (of osmolarity 393, 512 and 750 mOsm/L respectively) into the brachial arteries of control subjects and reported vasodilatation. However, they used jet infusions at a rate of 8.2 ml/min, which is much higher than our rate of 1 ml/min.

Bank et al, (1992) infused saline of osmolality 280 mOsm/Kg, 480 mOsm/Kg and 660 mOsm/kg into brachial arteries of control subjects and those with congestive cardiac failure. Once again the rate of infusion used (5ml/min for 2.5 minutes) was much higher than our infusion rate. However, they demonstrated that in the normal subjects, hyperosmolal infusions of 480 and 660 mOsm/Kg increased forearm blood flow by 3.12 ± 0.4 and 6.8 ± 0.67 ml/min/dL forearm volume respectively, both $p < 0.001$ compared with isosmolal infusions. In contrast, the effect on patients with cardiac failure was less though significant as compared with isotonic infusions.

It is fascinating to postulate mechanisms by which hyperosmolar solutions could mediate vasodilation:

- 1 Dehydration of cell wall structures or surrounding tissue leading to a passive increase in vascular lumen. To support this hypothesis a study by Overbeck et al (1961) demonstrated that dog forelimb weight decreases during intra-brachial arterial administration of hypertonic solutions in the face of reduced vascular resistance, indicating movement of fluid from the extravascular to the intravascular space and extraluminal dehydration.
- 2 Inhibition of smooth muscle myogenic tone. There are reports indicating that hypertonicity produces negative chronotropic and inotropic effects on isolated vascular smooth muscle (Mellander et al, 1967).
- 3 Sympathetic inhibition.
- 4 Electrolyte shifts (Haddy, 1960). This is perhaps unlikely as there is similarity of the vascular responses to equally hypertonic dextrose and saline in the limbs of man (Overbeck and Grega, 1970). However there is a possibility that an increase in viscosity produced by hypertonic dextrose and not by hypertonic saline may have masked a difference in effect. A more conclusive statement about specific vascular effects of the sodium ion might be made if you could observe response to changes in the concentration of the sodium ion alone in the absence of possible interaction with changes in plasma osmolality, but unfortunately it is impossible to increase plasma sodium significantly without increasing plasma tonicity unless some other plasma constituent is simultaneously

removed and such removal might also produce vasoactive effects or interaction.

- 5 Changes in blood viscosity. This would be due to reduced concentration and size and increased aggregation or deformity of red blood cells.
However the study of Overbeck and Grega (1970) showed no alteration in haematocrit.
- 6 Changes in plasma or cellular concentrations of vasoactive chemicals.
Such mechanisms remain to be studied.

In order to test the hypothesis that the hyperosmolarity of NAC solutions could account for the vasodilation in cirrhotic subjects and normal controls an experimental design for a further forearm blood flow study is proposed. The study group would consist of 6 control subjects and 6 patients with cirrhosis (with a spread of Child's Pugh gradings). Each subject would have to attend on two occasions:

- 1 on one occasion they would be given a cumulative incremental dose of NAC (1mg/min, 3mg/min, 10mg/min, 30 mg/min, 100 mg/min and all at infusion rates of 1ml/min), for 6 minutes each.
- 2 on the other occasion they would receive incremental increases in osmolarity of NaCl solutions for six minutes each, equivalent to the osmolarities of the NAC infusions:

Sodium chloride 1.8% for infusion has an osmolarity of 616 mOsm/L and Sodium chloride 2.7% has an osmolarity of 924 mOsm/L. Therefore, the following method of making up desirable osmolarities of NaCl in the 50 ml syringes used for forearm blood flow studies is proposed:

(Total mOsm contribution = 924)	2.7% NaCl (highest possible)	
	8 ml of 0.92 mOsm/ml	42 ml of 0.62 mOsm/ml
(Total mOsm contribution = 670)	7.36 +	26.04 in 50 ml ie 0.67 mOsm/ml
	20 ml of 0.62 mOsm/ml	30 ml of 0.31 mOsm/ml
(Total mOsm contribution = 430)	12.4 +	9.3 in 50 ml ie 0.434 mOsm/ml
	7 ml of 0.62 mOsm/ml	43 ml of 0.31 mOsm/ml
(Total mOsm contribution = 350)	4.34 +	13.33 in 50 ml ie 0.353 mOsm/ml
	2 ml of 0.62 mOsm/ml	48 ml of 0.31 mOsm/ml
(Total mOsm contribution = 320)	1.24 +	14.88 in 50 ml ie 0.322 mOsm/ml

All solutions would be infused at a rate of 1 ml/minute

NOT ALL VASODILATORS ARE THE SAME

It is fascinating that both N-acetylcysteine and Isosorbide-5-mononitrate are vasodilators yet appear to have differing haemodynamic actions in other respects in patients with cirrhosis.

For example Is-5-Mn causes reduced cardiac index (Hayes et al, 1988) but NAC increases it (Section III). NAC has no effect on estimated liver blood flow but Is-5-Mn has been shown to reduce liver blood flow (Hayes et al, 1988). Both vasodilate; for Is-5-Mn this results in a fall in mean arterial blood pressure that is dose-dependent (Section II) but for NAC there is no change in overall blood pressure.

NAC has no effect on mean hepatic venous pressure gradient but either 10 mg Is-5-Mn or 40 Mg Is-5-Mn were capable of producing a marked sustained reduction in HVPG. Neither Is-5-Mn or NAC appeared to have significant action on WHVP.

Thus not all vasodilators are equal and it may not be possible to predict the haemodynamic effects of one from knowledge of another.

Such haemodynamic differences between different vasodilators may result from:

- i) different molecular or subcellular mechanisms of action
- ii) actions on different vascular beds
- iii) activation of different reflex mechanisms, for example, the marked blood pressure fall in response to Is-5-Mn triggers splanchnic reflex vasoconstriction, which lowers the HVPG.

- iv) shifts in central blood volumes may alter the ability to respond to vasodilators - this may not show up in local vascular studies, such as section IV, but shows up with systemic haemodynamic studies in vivo (such as Section III) and points to the importance of such studies.
- v) hyperosmolar effects

Also by contrast to nitrate action is the effect of theophylline in patients with cirrhosis. Theophylline is an adenosine receptor antagonist and at a dose of 6mg/kg induced a significant increase in heart rate and reduction of right atrial pressure, stroke volume and systolic arterial pressure. In contrast, oxygen consumption or sympathoadrenal activity and all other haemodynamic values (including azygos blood flow) were unaltered. Thus theophylline causes a decrease in stroke volume which lowers systolic arterial pressure but, unlike nitrates, does not have a reflex vasoconstrictor effect on splanchnic or systemic circulation (Moreau et al, 1992).

THE PHARMACOKINETICS OF N-ACETYLCYSTEINE

A new method has recently been described for detection of NAC using reductive cleavage with tributylphosphine and post-HPLC column derivatisation with omicron-phthalaldehyde (Gabard and Mascher, 1991). Using this method, endogenous average concentrations of 0.08 micromol were measured in 10 volunteers participating. After a single oral dose of exogenous NAC (200mg) the drug was detected in plasma for up to 12h after administration; the C_{max} values were up to 20 times the endogenous levels. The sensitivity of this method,

though of no value to our intravenous pharmacokinetic study (where the sensitivity of the assay was adequate to follow drug concentrations for up to 10 hours), should enable the behaviour of smaller doses of NAC after oral administration to be properly described and bioavailability studies to be performed (Gabard and Mascher, 1991).

More interestingly perhaps this method could be used to assess if endogenous NAC concentrations were higher in patients with cirrhosis; this might account for the apparent lack of tolerance that these individuals show to nitrates. NAC concentrations have been found in the urine of patients with chronic liver disease (Martennson et al, 1992). The fact that the Cmax was not significantly different between our control and patient groups in Section V rules out the possibility that endogenously high levels are responsible for the pharmacokinetic differences observed between patients and controls. From the above we would anticipate that the endogenous NAC concentrations would be 1/60th of that achieved by intravenous dosage in our study (Section V).

We have demonstrated that the pharmacokinetics of N-acetylcysteine are altered in chronic liver disease. Our assay measures TOTAL plasma NAC i.e. total oxidised and reduced NAC, and NAC bound to other thiols and proteins.

Borgstroem et al, 1986 and 1990 method assayed NAC in deproteinised plasma, not total NAC. The variety of forms in which NAC is found make data comparison with other studies difficult. In addition, administration of NAC may displace homocysteine and cysteinylglycine from their protein sites by

disulphide interchange reactions. This leads to formation of mixed low-molecular weight cysteine and NAC disulphides with high renal clearance (Hultberg et al, 1994). Thus studying the kinetics of NAC is very difficult indeed.

Despite this the normal control results were broadly comparable in terms of area under the curve and estimated half-life of the drug between previous studies ours (Section V). Borgstroem however found that the kinetics conformed to a triphasic pattern whereas we found all our data fitted a biphasic (2 compartment model). Clearance was impaired and thus the area under the curve was different in those with cirrhosis (Section V). The volume of distribution at steady state was consistent with a distribution mainly to extracellular water. The volume of distribution at steady state was the same in both our control and cirrhotic patient groups and the control group values were similar to previous reports.

If hepatic flow were estimated 650 ml/min, the hepatic extraction ratio could be calculated from previous studies as 0.26 for NAC i.e. NAC is a drug with a low extraction ratio. In cirrhosis drugs with a high extraction ratio tend to have high peak values but normal half-lives whereas low extraction drugs have normal peak values but longer half-lives. Hepatic vein catheter studies (Pessayre et al, 1978) would allow us to calculate the hepatic extraction of N-acetylcysteine directly and would also allow the estimation of liver blood flow by the ICG method which would allow an estimation of the importance of flow to altered hepatic metabolism to be established.

The data in this study supports the proposal that shunting is not a very important factor in NAC pharmacokinetics as there is reduced clearance but not a high C_{max}.

Implications of the finding that the pharmacokinetics of N-acetylcysteine is altered in chronic liver disease for the studies reported in this text

The implications of the finding that the pharmacokinetics of N-acetylcysteine are altered in chronic liver disease are that haemodynamic comparison with normal controls is difficult as it would be difficult to model an equivalent dosage regime for patients with liver disease.

The results seen in the forearm blood flow studies (Section V) were the same in patients with chronic liver disease as normal controls but the forearm blood flow model aims to evaluate only the local haemodynamic actions of drugs in the forearm, and we would not necessarily expect altered metabolism of the drug for example by the liver in cirrhosis to have a significant impact on this.

The clinical implications of the altered pharmacokinetics of N-acetylcysteine in chronic liver disease

The study described in Section V may however have clinical implications in the treatment of paracetamol poisoning in patients with underlying cirrhosis. Bray et al (1991) have recently shown that chronic alcohol intake above suggested safe drinking limits is an adverse prognostic feature in cases of severe paracetamol overdose. This effect is partly related to increased nephrotoxicity. A recent working group on paracetamol poisoning has therefore suggested that

patients with induced liver enzymes, including those who drink in excess of the currently recommended safe drinking limits, should be treated with NAC at lower plasma paracetamol levels than would normally be considered as a treatment threshold; thus more patients with chronic liver disease may be exposed to N-acetylcysteine therapy than were seen in the past.

A recent study has demonstrated that acute ethanol administration reduces the antidote effect of N-acetylcysteine after acetaminophen overdose in mice (Dalhoff et al, 1991). In the NAC group the survival rate was 85%. This rate was significantly reduced to 43% in the NAC + ethanol group ($p = 0.0001$).

Perhaps the dosage schedule for intravenous NAC requires modification for patients with chronic liver disease in the light of the findings reported in this thesis as both the pharmacokinetics and systemic haemodynamics appear to be altered in the presence of liver disease.

Prescott and colleagues found that "adverse reactions invariably occur early when plasma concentrations are at their highest, and liver damage was prevented just as effectively at the lowest as at the highest C_{max} " (Ho and Beilin, 1983; Mant et al, 1984; Donovan et al, 1987; Prescott et al, 1989) i.e high plasma concentrations of NAC are likely to predispose to adverse drug reactions including flushing, urticaria, hypotension and bronchospasm.

Typically, these "anaphylactoid" reactions occur shortly after starting therapy at a time when the concentration of NAC is at the highest. However it should be noted in Section V that the C_0 values were not significantly different between

patients with liver disease and controls and so an increase in anaphylactoid reactions would not be expected in chronic liver disease patients.

The dosage regimen for treatment of paracetamol poisoning with intravenous NAC was originally chosen on an arbitrary basis; its efficacy was known to fall off rapidly when treatment was delayed beyond 8 to 10 hours and the aim was to give the highest tolerable dose for a short period in the hope of reversing the events leading to hepatic necrosis at a critical stage (Prescott et al, 1977). This regimen, although of proven value in the treatment of paracetamol poisoning, is unlikely to be the most optimal one.

The results in Sections III and V imply that we should be more vigilant for adverse effects to N-acetylcysteine in those with chronic liver disease and we should consider modification of the current paracetamol overdose treatment regime with N-acetylcysteine for those with chronic liver disease. The last suggestion would be difficult as a study using "dose-response" to NAC in paracetamol poisoning would be unethical and whilst it is reasonably simple to model NAC kinetics it is not easy to model the hepatoprotective effect of the drug by as yet an unknown mechanism (sulphydryl donation, free radical scavenging and a variety of other mechanisms of action have all been proposed).

CONCLUSIONS OF “HAEMODYNAMIC STUDIES IN CIRRHOSIS”

- Either 10 mg or 40 mg bd oral Is-5-Mn were effective in reducing HVPG and there was no evidence of tolerance when a nitrate free interval was used in patients with cirrhosis. The 40 mg dose caused greater systemic haemodynamic change than the 10 mg dose. The azygos vein blood flow response to nitrate appeared to relate to initial flow rather than to dose of nitrate used or severity of liver disease; this requires a prospective study to confirm the observation.

Further studies need to identify if the greater haemodynamic disturbance of the 40 mg dose of nitrate compared with the 10 mg dose translates into greater renal disturbance.

Multicentre large randomised clinical trials are needed to evaluate the clinical efficacy of Is-5-Ms alone and in combination with propranolol in preventing variceal bleeding.

Intravenous N-acetylcysteine administration increased cardiac index and tissue O₂ delivery in patients with cirrhosis but tissue O₂ consumption was not increased. NAC had no haemodynamic action on the portal system. NAC was a potent peripheral vasodilator in patients with cirrhosis and normal controls.

Further studies are required to determine if this is due to its hyperosmolality. As NAC is hypertonic it is vital that one uses hypertonic solutions as controls for both local and systemic haemodynamic studies in future, ie 5% dextrose was not, with hindsight, the best possible control solution for the study in Section III.

Further studies are also required into the mechanism of action of NAC as a positive inotrope. The development of a variety of specific inhibitors (eg L-NMMA) should make this task easier.

The time course of the haemodynamic action of NAC requires further investigation as NAC infusions are used over many hours in clinical medicine. It would be particularly interesting to discover if the vasodilatation and positive inotropic action persist throughout the time of infusion.

- The clearance of NAC was impaired in patients with chronic liver disease. This lends support to the hypothesis that NAC is cleared by the liver.

Further studies are needed to evaluate if the clearance of NAC is impaired in patients with fulminant hepatic failure and if modification of the dosage regime for such patients is required. Observations for adverse haemodynamic effects in patients with impaired liver function who are receiving NAC treatment should be made.

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Appendices

Appendix Ia PATIENT DETAILS - 10 mg Is-5-Mn STUDY GROUP

Patient No.	Age (yrs)	Sex	Ascites	Encephalopathy	Bilirubin $\mu\text{mol/L}$	ALT IU/L	GGT IU/L	Albumin g/L	PTR secs (ratio)	Pugh score	Aetiology
1	49	F	moderate	no	202	60	359	33	23/12 (1.92)	C (12)	ALC
2	70	M	no	no	32	25	204	38	12/12 (1.00)	A (5)	ALC
3	51	F	moderate	no	77	61	62	28	14/12 (1.17)	B (9)	PBC
4	65	F	no	no	106	26	315	32	17/12 (1.42)	B (9)	ALC
5	39	F	slight	grade 1	24	55	625	32	13/12 (1.08)	B (8)	ALC
6	42	M	moderate	no	25	34	170	24	14/12 (1.17)	B (9)	ALC
7	60	F	no	no	12	32	258	40	14/12 (1.17)	A (5)	PBC
8	67	F	no	no	10	19	36	42	12/12 (1.00)	A (5)	ALC
9	63	F	marked	no	83	18	156	31	16/12 (1.33)	C (10)	ALC
10	69	M	marked	no	41	22	92	44	14/12 (1.17)	B (8)	ALC
11	74	M	no	no	36	57	216	33	12/12 (1.00)	B (7)	ALC
12	64	F	marked	no	21	26	43	36	19/12 (1.58)	B (9)	ALC

Appendix Ib

PATIENT DETAILS - 40 mg Is-5-Mn STUDY GROUP

Patient No.	Age (yrs)	Sex	Ascites	Encephalopathy	Bilirubin $\mu\text{mol/L}$	ALT IU/L	GGT IU/L	Albumin g/L	PTR secs (ratio)	Pugh score	Aetiology
1	49	M	no	no	15	20	146	42	12/12 (1.00)	A (5)	PBC
2	54	F	marked	grade 1	136	40	71	29	16/12 (1.33)	C (11)	ALC
3	54	M	slight	no	22	30	271	39	12/12 (1.00)	A (6)	ALC
4	40	F	marked	grade 1	27	12	436	22	15/12 (1.25)	C (10)	SLC
5	64	M	slight	grade 1	18	21	20	28	17/12 (1.42)	B (9)	ALC
6	61	F	grade 1	grade 1	41	18	25	26	18/12 (1.50)	C (12)	ALC
7	54	M	no	no	52	46	1914	28	14/12 (1.17)	B (8)	ALC
8	52	M	no	no	28	16	26	30	16/12 (1.33)	A (6)	ALC
9	63	M	marked	grade 1	111	81	572	32	16/15 (1.07)	C (11)	ALC
10	71	M	slight	no	26	19	65	31	16/12 (1.33)	B (7)	ALC
11	49	M	slight	no	140	68	264	37	14/14 (1.00)	B (8)	SBC
12	54	M	slight	no	9	33	38	40	13/12 (1.08)	A (6)	ALC
13	63	M	no	no	30	20	206	35	15/12 (1.25)	A (6)	ALC

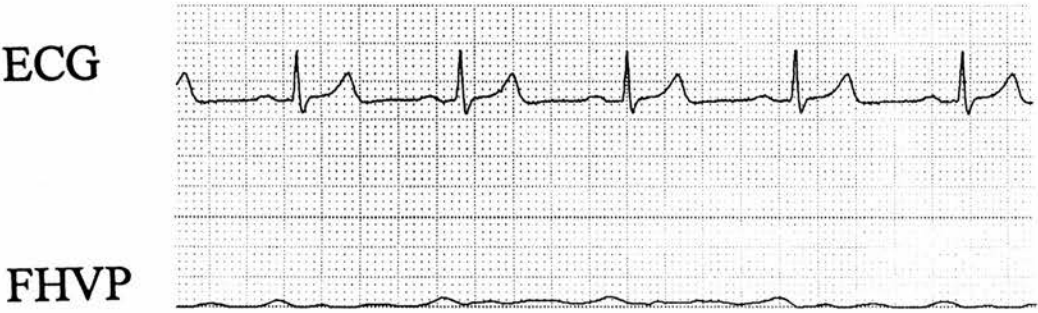
Appendix II

BASELINE CHARACTERISTICS OF THE 10 mg AND 40 mg Is-5-Mn STUDY GROUPS

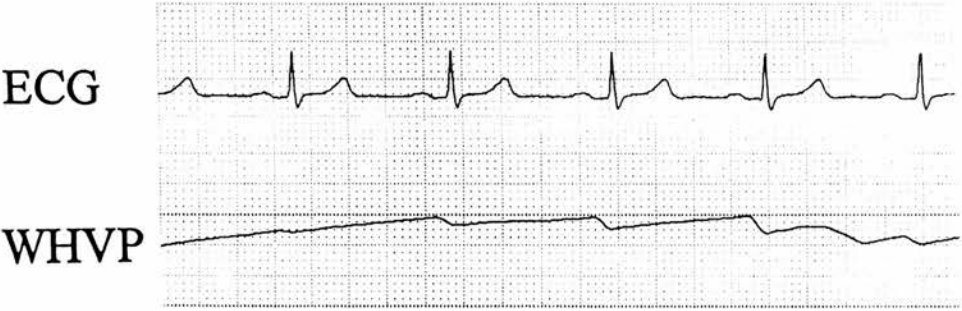
Variable	10 mg Is-5-Mn group	40 mg Is-5-Mn group	p value
Number in group	n = 12	n = 13	
Mean age \pm SEM	59.4 \pm 3.3	56.0 \pm 2.3	p > 0.32 (NS)
Age range (yrs)	42-74	40-71	
Sex	8 female: 4 male	10 male: 3 female	
Aetiology	10 ALC: 2 PBC	11 ALC: 1 PBC: 1 SBC	
Child's-Pugh Score	8.0 \pm 0.6	8.1 \pm 0.6	p > 0.5 (NS)
Serum bilirubin (umol/L)	55.8 \pm 16.0	50.4 \pm 12.9	p > 0.05 (NS)
Serum albumin (g/L)	34.4 \pm 1.7	32.2 \pm 1.7	p >> 0.5 (NS)
PTR ratio	1.25 \pm 0.08	1.20 \pm 0.05	p >> 0.5 (NS)
Serum alanine aminotransferase (u/L)	36.2 \pm 4.9	32.6 \pm 5.9	p >> 0.5 (NS)
Number with ascites	7	9	

Appendix III

Free hepatic venous pressure trace

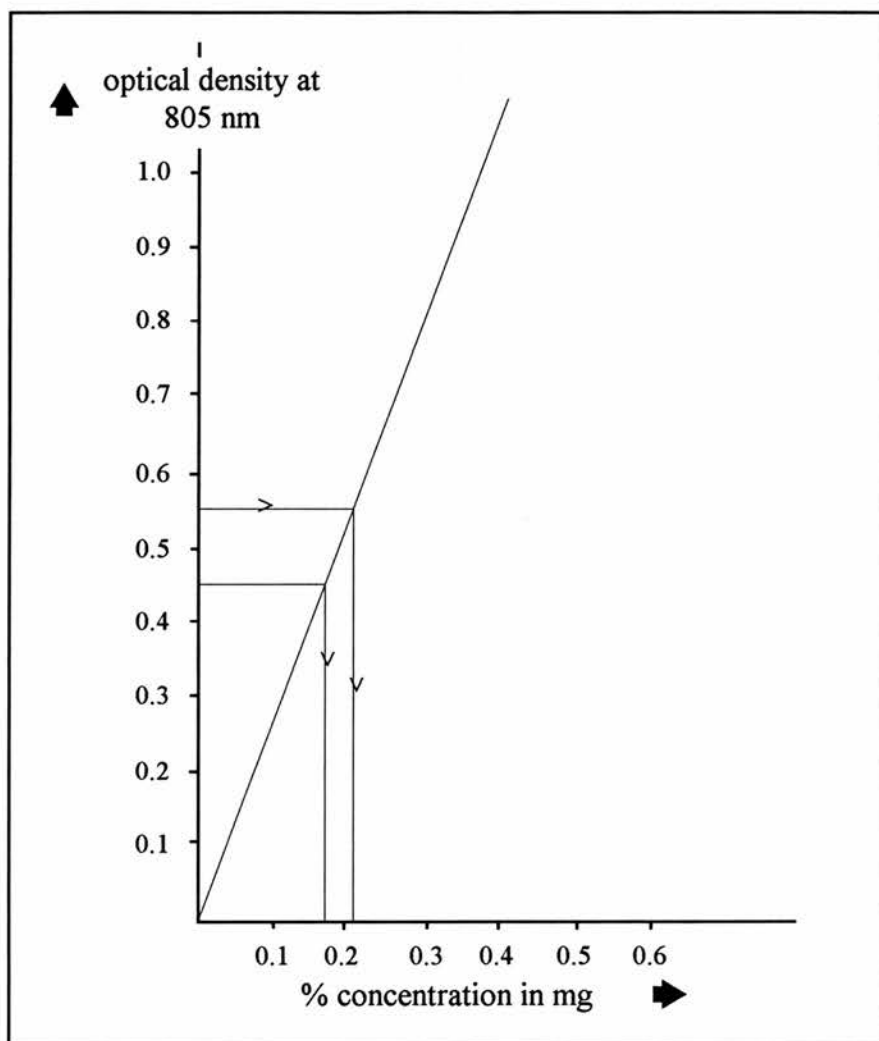


Wedged hepatic venous pressure trace



ICG CONCENTRATIONS IN SERUM AS MEASURED BY OPTICAL DENSITY AT 805 nm

Appendix IV



Mean optical density of peripheral venous blood = 0.550

Mean optical density of hepatic venous blood = 0.455

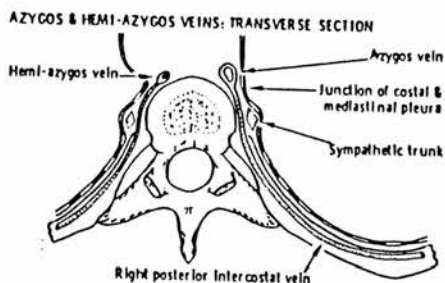
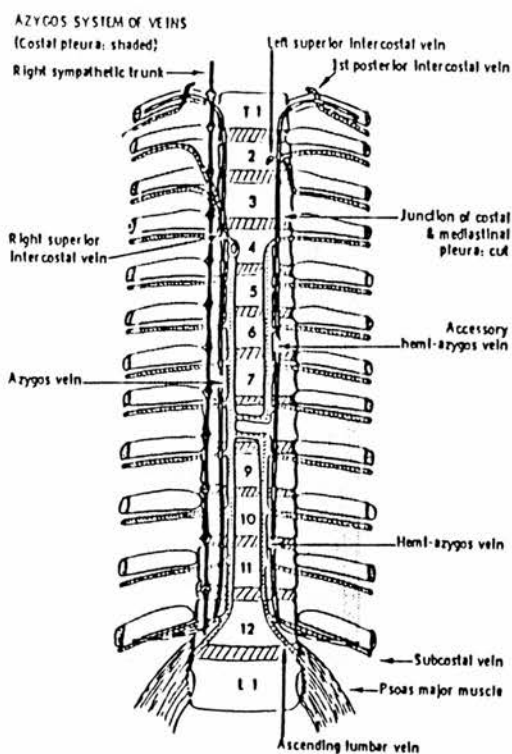
Patients' haematocrit = 0.414

Rate of infusion of ICG = 0.25 mg/min

Therefore: estimated liver blood flow

$$= \frac{0.25}{0.0004 \times (1-0.414)}$$

$$= 1066 \text{ ml/min}$$



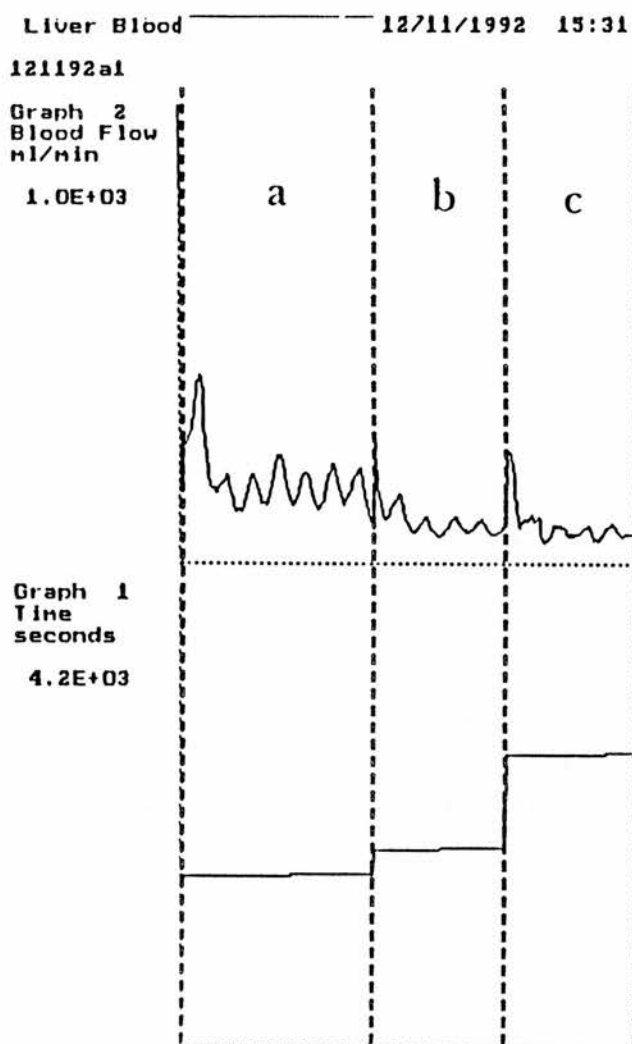
Appendix V

The azygos vein

The azygos vein is formed on the right side of the anterior aspect of the twelfth thoracic vertebral body by the union of the right subcostal vein and the right ascending lumbar vein which emerges from the upper part of the right psoas major muscle.

The vessel passes directly up the right side of the anterior aspect of the vertebral column, covered on its lateral side by the most posterior part of the mediastinal pleura. In front of the fourth thoracic vertebral body it turns forwards towards its junction with the superior vena cava and the anterior part of the mediastinum; it is at this point that the azygos catheter is introduced, via the superior vena cava.

The azygos vein receives all the right posterior intercostal veins except the first. The second, third and fourth are received through a common trunk called the right superior intercostal vein, and other individually. These tributaries leave their respective intercostal spaces, pass behind the sympathetic trunk and incline forwards across the lateral aspects of the vertebral bodies. The azygos vein is also joined by visceral tributaries from the right extrapulmonary bronchi and from the oesophagus.



Appendix VIa

A sample azygos blood flow recording

a = flow prior to 40mg Is-5-Mn

b = flow at 30 minutes after ingestion of 40mg Is-5-Mn

c = flow at 60 minutes after ingestion of 40mg Is-5-Mn

The baseline variations seen in the trace reflect the effect of respiration on the azygos blood flow.

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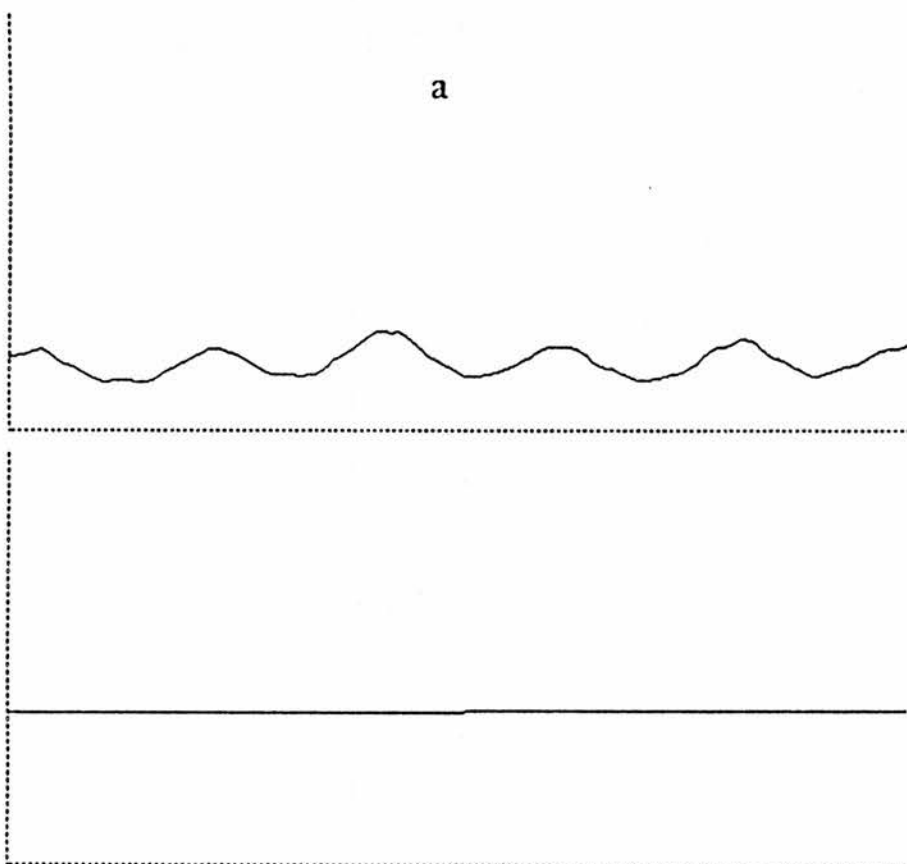
Graph 2
Blood Flow
ml/min

1.0E+03

a

Graph 1
Time
seconds

4.2E+03



Appendix VIb

Before blood flow can be measured, each trace must be expanded (a).

This also allows the artifact of the first few seconds of the recording to be omitted and the figures taken from the steady part of the trace.

Key to appendices VII - X

- A = heart rate increase (bpm) from baseline value to t=60, 1st study
- B = heart rate increase (bpm) from baseline value 1st study to baseline 2nd study
- C = heart rate increase (bpm) from baseline value 1st study to t=60, 2nd study
- D = mean arterial blood pressure (mmHg) fall from baseline value to t=60, 1st study
- E = mean arterial blood pressure (mmHg) fall from baseline value 1st study to baseline 2nd study
- F = mean arterial blood pressure (mmHg) fall from baseline value 1st study to t=60, 2nd study
- G = Wedged Hepatic Venous Pressure (mmHg) fall from baseline value to t=60, 1st study
- H = Wedged Hepatic Venous Pressure (mmHg) fall from baseline value 1st study to baseline 2nd study
- I = Wedged Hepatic Venous Pressure (mmHg) fall from baseline value 1st study to t=60, 2nd study
- J = Free Hepatic Venous Pressure (mmHg) fall from baseline value to t=60, 1st study
- K = Free Hepatic Venous Pressure (mmHg) fall from baseline value 1st study to baseline 2nd study
- L = Free Hepatic Venous Pressure (mmHg) fall from baseline value 1st study to t=60, 2nd study
- M = Hepatic Venous Pressure Gradient (mmHg) fall from baseline value to t=60, 1st study
- N = Hepatic Venous Pressure Gradient (mmHg) fall from baseline value 1st study to baseline 2nd study
- O = Hepatic Venous Pressure Gradient (mmHg) fall from baseline value 1st study to t=60, 2nd study

P = Azygos vein blood flow reduction (mL/min) from baseline value to t=60, 1st study

Q = Azygos vein blood flow reduction (mL/min) from baseline value 1st study to baseline 2nd study

R = Azygos vein blood flow reduction (mL/min) from baseline value 1st study to t=60, 2nd study

S = serum albumin in g/L

T = prothrombin time (ratio)

U = serum alanine aminotransferase in U/L

V = serum bilirubin in $\mu\text{mol/L}$

	A	B	C	D	E
1	3	0	6	0	3.3
2	0	2	-1	1.7	-7.3
3	7	-3	7	6	-4
4	-1	-45	-42	16	11.3
5	5	20	22	2	0
6	4			5	
7	-6	-16	-11	0	-4
8	5			3.4	
9	8	6	7	-7	8
10	6	11	2	2	3.4
11	-1	-4	-4	16.6	10
12	-3			15	

	F	G	H	I	J
1	3.3	3	-8	-3	-1
2	-20	12	6	6	1
3	-2	1	4	10	-4
4	8	10	7	6	2
5	3.4	-1	1	-1	0
6		0			0
7	1.3	1	-3	-2	0
8		-3			0
9	13.3	3	7	8	-1
10	13.4	10	10	11	2
11	13.3	4	14	11	1
12		-5			-5

	K	L	M	N	O
1	-3	-3	4	-5	0
2	-3	-3	11	9	9
3	-6	3	5	10	7
4	0	-2	8	7	8
5	-2	0	-1	3	-1
6			0		
7	-3	-2	1	0	0
8			3		
9	-5	-3	4	12	11
10	-5	-4	8	15	15
11	13	11	3	1	0
12			0		

	P	Q	R	S	T
1	357	488	481	33	1.92
2	-17	33	238	38	1
3	152	175	73	28	1.17
4	-8	-1052	-986	32	1.42
5	-33.9	-183.5	-199.5	32	1.08
6	-4			24	1.17
7	150	23	27	40	1.17
8	-62			42	1
9	53	-530	-1037	31	1.33
10	97	-85	-526	44	1.17
11	77	-144	-236	33	1
12	-113			36	1.58

	U	V
1	60	202
2	25	32
3	61	77
4	26	106
5	55	24
6	34	25
7	32	12
8	19	10
9	18	83
10	22	41
11	57	36
12	26	21

Appendix VII

Change in haemodynamic measurements from baseline values in response to 10mg Is-5-Mn.

	A	B	C	D	E
A	1.000				
B	(0.686)	1.000			
C	(0.832)	(0.778)	1.000		
D	-0.253	-0.336	-0.287	1.000	
E	-0.034	-0.218	-0.265	0.359	1.000
F	0.206	0.218	-0.021	0.177	(0.824)
G	-0.191	-0.127	-0.551	0.191	(0.254)
H	0.088	0.000	-0.265	0.515	0.605
I	0.338	0.101	0.000	0.530	0.346
J	-0.468	-0.110	-0.643	0.444	0.387
K	(-0.727)	-0.349	-0.483	0.459	0.419
L	-0.278	-0.417	-0.017	0.622	0.056
M	(0.148)	-0.059	-0.274	0.127	-0.025
N	(0.686)	0.433	0.276	0.034	0.059
O	0.417	0.170	-0.111	-0.154	0.153
P	0.050	-0.317	-0.033	-0.160	-0.092
Q	0.025	-0.000	0.151	-0.227	(0.686)
R	-0.184	-0.033	0.117	-0.160	(0.745)
S	-0.382	0.084	-0.361	-0.215	-0.092
T	0.184	-0.213	-0.009	-0.352	0.368
U	-0.159	-0.317	0.151	0.378	-0.201
V	0.351	-0.150	0.059	-0.008	0.527

	F	G	H	I	J
F	1.000				
G	0.182	1.000			
H	(0.676)	0.585	1.000		
I	0.519	0.357	(0.882)	1.000	
J	0.417	(0.700)	0.587	0.291	1.000
K	0.150	0.147	0.158	-0.185	0.502
L	-0.180	-0.474	0.021	0.124	-0.216
M	-0.105	(0.817)	0.346	0.322	0.385
N	0.310	(0.321)	0.561	(0.681)	0.153
O	0.298	(0.670)	0.545	0.521	0.310
P	-0.092	-0.211	-0.310	-0.050	-0.449
Q	-0.612	-0.093	-0.544	-0.244	-0.449
R	(-0.795)	-0.152	(-0.695)	-0.496	-0.390
S	0.134	0.360	-0.057	-0.198	0.443
T	0.158	-0.086	-0.316	-0.378	-0.242
U	-0.418	-0.498	-0.444	-0.202	-0.475
V	0.243	0.253	0.075	0.034	-0.161

	K	L	M	N	O
K	1.000				
L	0.426	1.000			
M	-0.300	-0.502	1.000		
N	-0.604	-0.366	0.563	1.000	
O	-0.528	(-0.667)	(0.786)	(0.831)	1.000
P	-0.443	-0.051	-0.050	-0.233	-0.051
Q	-0.417	-0.111	0.126	-0.267	-0.153
R	-0.102	0.051	0.008	-0.500	-0.424
S	0.103	-0.560	0.105	-0.276	0.060
T	-0.243	-0.374	0.094	-0.077	0.156
U	0.145	(0.672)	-0.361	-0.583	(-0.729)
V	-0.170	-0.264	0.395	0.117	0.322

	F	Q	R	S	T
F	1.000				
Q	(0.667)	1.000			
R	0.383	(0.900)	1.000		
S	0.285	0.368	0.293	1.000	
T	0.451	-0.026	-0.136	0.017	1.000
U	0.483	0.533	0.617	-0.151	-0.017
V	0.350	0.000	-0.133	-0.134	(0.775)

Appendix VIII

	U	V
U	1.000	
V	0.067	1.000

Matrix of Spearmans' Rank Correlation between changes in haemodynamic variables and plasma bilirubin, albumin, ALT and PTR in the 10 mg Is-5-Mn group. (Statistically significant values at the 95% level are ringed)

	A	B	C	D	E
1	10	16	26	5	-7.4
2	-1	30	30	-1.7	-5
3	6	3	10	15.6	10.6
4	18	11	23	0.7	10
5	5	5	-4	-1	-20
6	15	2	2	3.3	29.3
7	15	11	2	8.6	-4.7
8	17			0.7	
9	10			14.7	
10	8	8	-6	29.4	0.7
11	2	11	18	6	3.3
12	11	-6	-6	5.3	-2.7
13	17	2	19	4.4	-3.3

	F	G	H	I	J
1	1.3	6	9	10	1
2	-1.7	-1	8	5	-2
3	17.3	1	1	4	1
4	5	-2	3	3	0
5	11.7				2
6	23.3	3	1	1	-1
7	-19.4	1	1	2	-1
8		-1			-1
9		8			0
10	10.7	10	8	12	-2
11	7.3	3			1
12	4.7	4	1	5	3
13	-4	2	8	6	-3

	K	L	M	N	O
1	-1	-2	5	10	12
2	3	1	1	5	4
3	1	1	0	0	3
4	0	-1	-2	3	4
5	9	6			
6	-1	-3	4	2	4
7	-2	-3	2	3	5
8			0		
9			8		
10	10	11	12	-2	1
11	0	-1	2		11
12	-1	-2	1	2	3
13	-1	-1	4	8	6

	P	Q	R	S	T
1	129	-91	-4	42	1
2	51	1084	1353	29	1.33
3	8	273	-244	39	1
4	-2	394.6	392.7	22	1.25
5	211	53	-474	28	1.42
6	146	372	360	26	1.5
7	23	309	444.5	28	1.17
8	-177			30	1.33
9	-201.3			32	1.07
10	71	-229	-113	31	1.33
11	298	-40	-42	37	1
12	20.5	-126.5	-768.5	40	1.08
13	-34	-278	-362	35	1.25

	U	V
1	20	15
2	40	136
3	30	22
4	12	27
5	21	18
6	18	41
7	46	52
8	16	28
9	81	111
10	19	26
11	68	140
12	33	9
13	20	30

Appendix IX

Change in haemodynamic parameters from baseline values in response to 40 mg Is-5-Mn.

	A	B	C	D	E
A	1.000				
B	-0.321	1.000			
C	-0.093	(0.695)	1.000		
D	-0.268	-0.286	(-0.681)	1.000	
E	0.218	-0.580	-0.462	0.133	1.000
F	-0.151	-0.353	-0.319	0.150	(0.833)
G	-0.189	-0.342	-0.527	0.544	-0.075
H	-0.178	0.536	0.559	-0.213	-0.612
I	-0.378	0.127	0.063	0.293	-0.477
J	-0.076	-0.153	-0.140	0.211	0.127
K	-0.581	0.255	0.132	0.035	0.218
L	-0.528	0.205	0.150	0.170	0.059
M	-0.089	-0.025	-0.246	0.319	-0.269
N	0.245	0.453	(0.758)	-0.588	(-0.740)
O	0.402	0.343	0.597	-0.426	-0.587
P	-0.385	0.244	-0.109	0.033	-0.083
Q	-0.075	0.538	0.471	-0.617	0.083
R	-0.050	(0.782)	0.479	-0.483	-0.233
S	-0.444	-0.168	-0.034	0.433	-0.400
T	0.119	-0.017	-0.089	-0.414	0.287
U	-0.445	0.173	0.008	0.192	-0.561
V	0.092	0.370	0.303	-0.483	-0.100

	F	G	H	I	J
F	1.000				
G	0.209	1.000			
H	-0.310	0.249	1.000		
I	-0.184	0.580	(0.779)	1.000	
J	0.304	0.093	-0.391	-0.182	1.000
K	0.435	-0.052	0.324	0.406	-0.220
L	0.186	-0.051	0.415	0.579	-0.305
M	-0.059	(0.819)	0.492	0.532	-0.391
N	(-0.698)	-0.215	0.568	0.148	-0.162
O	(-0.621)	-0.141	0.389	-0.047	-0.159
P	0.300	0.519	0.133	0.017	0.034
Q	0.100	(-0.711)	-0.222	(-0.653)	0.076
R	-0.217	-0.544	0.089	-0.418	-0.279
S	-0.117	0.569	0.284	(0.653)	0.405
T	0.203	-0.055	0.058	-0.165	(-0.701)
U	-0.619	-0.151	-0.196	-0.017	0.072
V	-0.333	-0.544	-0.027	-0.435	(-0.717)

	K	L	M	N	O
K	1.000				
L	(0.921)	1.000			
M	-0.052	-0.068	1.000		
N	-0.413	-0.291	0.076	1.000	
O	(-0.638)	-0.519	0.202	(0.914)	1.000
P	0.017	-0.254	0.588	-0.101	-0.009
Q	0.113	-0.144	-0.555	0.101	0.051
R	0.052	-0.127	-0.151	0.269	0.272
S	-0.044	0.136	0.269	0.134	0.043
T	0.291	0.099	0.238	-0.174	-0.185
U	-0.245	-0.102	-0.156	0.152	0.085
V	-0.035	-0.093	-0.042	0.227	0.272

	P	Q	R	S	T
P	1.000				
Q	0.200	1.000			
R	0.333	(0.833)	1.000		
S	-0.000	(-0.633)	(-0.667)	1.000	
T	0.312	0.279	0.388	(-0.692)	1.000
U	-0.075	0.042	0.109	0.326	-0.364
V	0.083	0.550	(0.750)	(-0.717)	(0.650)

Appendix X

	U	V
U	1.000	
V	0.184	1.000

Matrix of Spearman's Rank correlations between changes in haemodynamic variables and plasma bilirubin, albumin, ALT and PTR in the 40 mg Is-5-Mn group. (statistically significant values at the 95% level are ringed)

Appendix XI

N-acetylcysteine study patient details

Patient No.	Age (yrs)	Sex	Ascites	Encephalo pathy	Bilirubin (umol/L)	ALT (U/L)	GGT (U/L)	Albumin (g/L)	PTR ratio	Pugh Score	Aetiology
1	66	F	no	no	6	26	61	44	11/12	A (5)	PBC
2	52	F	moderate	no	100	20	30	29	21/12	C (11)	PBC
3	72	F	no	no	11	34	43	33	16/12	A (6)	Crypt
4	70	M	moderate	no	84	174	38	23	26/12	C (13)	ALC
5	43	M	slight	no	74	31	214	26	18/12	C (11)	ALC
6*	68	M	moderate	no	40	19	76	46	14/12	B (8)	ALC
7*	59	M	no	no	19	98	372	44	12/12	A (5)	ALC
8*	61	M	marked	no	31	15	87	32	15/12	B (8)	ALC
9	54	M	marked	no	71	30	90	35	14/12	C (10)	ALC
10*	74	M	marked	grade 1	51	27	76	30	16/12	C (10)	ALC
11*	57	M	no	no	43	24	80	40	15/12	A (6)	ALC
MEAN	61.5	-	-		48.2	45.3	106.1	34.7	-	8.5	-

SUMMARY OF FORMULAE USED IN HAEMODYNAMICS CALCULATIONS

Appendix XII

NAME	ABBREVIATION	UNITS	EQUATION	NORMAL RANGE (if available)
Body surface area	BSA	m ²	$3.207/10^4 \times Wt^{(0.7285 - 0.0188 \times \log Wt)} \times Ht^{0.3}$	
Mean arterial blood pressure	MABP or ABPm	mmHg	diastolic + 1/3 (systolic - diastolic BP)	
Cardiac index	CI	L/min/m ²	$\frac{CO}{BSA}$	2.5 - 4.0
Stroke volume	SV	mL	$\frac{CO}{HR} \times 1000$	
Systemic vascular resistance index	SVRI	dynes x sec/cm ⁵ x m ²	$79.96 \times \frac{(ABPm - CVP) \times BSA}{CO}$	1970 - 2390
Pulmonary vascular resistance index	PVRI	dynes x sec/cm ⁵ x m ²	$79.96 \times \frac{(PAPm - PAWP) \times BSA}{CO}$	225 - 315
Left ventricular stroke work index	LVSWI	g x m/m ²	$\frac{0.0136 \times ABPm \times SV}{BSA}$	50 - 62

Appendix XII contd

NAME	ABBREVIATION	UNITS	EQUATION	NORMAL RANGE (if available)
Oxygen content arterial blood	CaO ₂	mL/dL	$\frac{(1.34 \times \text{Hb} \times \text{SaO}_2) + (0.0031 \times \text{PaO}_2)}{100}$	17 - 20
Oxygen content mixed venous (PA) blood	CvO ₂	mL/dL	$\frac{(1.34 \times \text{Hb} \times \text{SvO}_2) + (0.0031 \times \text{PvO}_2)}{100}$	12 - 15
Arterial - venous oxygen content	avDO ₂	mL/dL	$\text{CaO}_2 - \text{CvO}_2$	4.2 - 5.0
O ₂ delivery		mL/min	$\text{CaO}_2 \times \text{CO} \times 10$	950 - 1150
O ₂ consumption		mL/min	$\text{avDO}_2 \times \text{CO} \times 10$	
Oxygen extraction ratio		%	$\frac{\text{CaO}_2 - \text{CvO}_2}{\text{CaO}_2}$	0.24 - 0.28

KEY: CO = cardiac output in L/min, Wt = body weight in grams, Ht = body height in cm, CVP = right atrial pressure in mmHg, PAPm = mean free pulmonary artery pressure in mmHg, PAWP = pulmonary artery wedge pressure in mmHg, SO₂ = saturation oxygen (%), PO₂ = partial pressure oxygen (kPa), a = arterial, v = venous.

Key to appendices XIII and XIV

A = serum bilirubin in $\mu\text{mol/L}$

B = serum albumin in g/L

C = prothrombin time (ratio)

D = heart rate increase (bpm) from baseline to t=30 minutes

E = fall in mean arterial blood pressure (mmHg) from baseline to t=30 minutes

F = fall in right atrial pressure (mmHg) from baseline to t=30 minutes

G = fall in pulmonary artery pressure (mmHg) from baseline to t=30 minutes

H = fall in pulmonary capillary wedge pressure (mmHg) from baseline to t=30 minutes

I = rise in cardiac index (L/min/m²) from baseline to t=30 minutes

J = reduction in femoral artery pO₂ (kPa) from baseline to t=30 minutes

K = reduction in femoral artery oxygen saturation (%) from baseline to t=30 minutes

L = increase in femoral vein pO₂ (kPa) from baseline to t=30 minutes

M = increase in femoral vein oxygen saturation (%) from baseline to t=30 minutes

N = reduction in pulmonary artery pO₂ (kPa) from baseline to t=30 minutes

O = reduction in pulmonary artery oxygen saturation (%) from baseline to t=30 minutes

P = reduction in free hepatic venous pressure (mmHg) from baseline to t=30 minutes

Q = increase in estimated liver blood flow (mmHg) from baseline to t=30 minutes

R = reduction in wedged hepatic venous pressure (mmHg) from baseline to t=30 minutes

Patient No	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	6	44	0.92	3	-2	0	2	2	0.41	-0.7	0	0.4	4.9	0.2	1.8			
2	100	29	1.75	-3	0	-1	1	1	-0.04	1.3	1.7	0.2	3.9	-0.2	-2.2			
3	11	33	1.33	6	-0.67	0	3	1	0.09	-0.5	-0.2	0	0	-0.4	-1	-2		
4	84	23	2.17	-2	-0.67	-1	2	-3	0.46	2.2	0	-0.7	-4	0.7	4.7			
5	74	26	1.5	3	2	0	2	2	0.28	-1.8	-1	0.5	-0.2	-0.3	-2.1			
6	40	46	1.17	-10	4	-1	-1	-1	0.5	0.6	0.7	0.3	4.5	-0.1	-3.5	0	246	-1
7	19	44	1	4	-4	1	2	-1	0.28	0.5	-1	0.1	4	-0.2	-7	0	89	-1
8	31	32	1.25	5	31	2	3	1	1.05	3.4	3	1.8	24.2	-0.1	-2.7	-1	-239	-2
9	71	35	1.17	2	-2	0	0	0	1.13	0.5	0.3	3.6	29.9	-0.3	-0.3	-2	410	-1
10	51	30	1.33	-2	0.67	0	1	0	0.54	1.6	0.4	0.6	9.7	0.2	-3.3	-2	-358	-2
11	43	40	1.25	4	-4	-1	-1	2	0.97	3	2.8	-0.6	0	0.4	-2.8	-2	579	-2

Appendix XIII

Change of haemodynamic parameters from baseline value after infusion of N-acetylcysteine

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
A	1																
B	-0.429	1															
C	0.319	-0.667	1														
D	-0.464	-0.232	-0.044	1													
E	-0.058	-0.29	0.397	-0.118	1												
F	-0.441	-0.441	-0.134	0.642	0.254	1											
G	-0.464	-0.493	-0.015	0.603	0.279	0.985	1										
H	0.294	-0.53	0.597	0.552	-0.015	-0.015	-0.015	1									
I	0.6	-0.543	0.0232	0.29	0.232	0.118	0.029	0.677	1								
J	-0.116	-0.435	0.794	0.426	0.456	0.075	0.147	0.746	0.29	1							
K	-0.086	-0.2	0.638	0.319	0.551	-0.088	-0.058	0.677	0.371	0.928	1						
L	0.429	-0.543	0.029	-0.058	0.58	0.441	0.377	0	0.657	-0.087	-0.029	1					
M	0.429	-0.543	0.029	-0.058	0.58	0.441	0.377	0	0.657	-0.087	-0.029	1	1				
N	0.058	-0.203	0.809	0	0	-0.433	-0.309	0.552	-0.116	0.735	0.609	-0.551	-0.551	1			
O	0.6	-0.543	0.232	0.29	0.232	0.118	0.029	0.677	1	0.29	0.371	0.657	0.657	-0.116	1		
P	0.833	0.679	-0.532	-0.016	0.204	0.207	0.204	-0.699	-0.679	-0.235	-0.123	-0.247	-0.247	-0.329	-0.679	1	
Q	-0.098	0.683	0.891	-0.396	-0.198	-0.101	-0.198	0.804	-0.293	0.891	-0.683	0.098	0.098	-0.792	-0.293	0.527	1
R	0.257	0.543	-0.377	0	-0.522	-0.618	-0.725	0.235	0.257	-0.203	0.029	-0.314	-0.314	-0.029	0.257	-0.185	0.293

Appendix XIV

Matrix of Spearmans' Rank Correlations between changes in haemodynamic variables and plasma bilirubin, albumin and prothrombin ratio in response to N-acetylcysteine.

(Statistically significant values at the 95% level are ringed)

Appendix XV

Patients details (cirrhotic group) in the forearm blood flow study

Patient no.	Age (years)	Sex	Aetiol	Ascites	Enceph	Bili (umol/L)	ALT (iu/L)	GGT (iu/L)	Albumin (g/L)	PTR ratio	Childs Pugh Score	Smoker ?
P-1	67	M	ALC	No	No	18	23	255	40	11/12	A(5)	Yes
P-2	58	F	PBC	No	No	19	48	546	45	11/12	A(5)	No
P-3	69	F	PBC	No	Grade 1	6	21	541	47	11/12	A(6)	No
P-4	55	M	ALC	No	No	24	18	71	35	16/12	B(7)	Yes
P-5	60	M	ALC	No	No	19	98	372	44	12/12	A(5)	Yes
P-6	55	M	ALC	No	No	8	112	103	36	13/12	A(5)	No
Mean	60.7 ± 2.5	4M:2F				15.7 ± 2.9	53.3 ± 17.0	314.7 ± 84.8	41.2 ± 2.0	1.93 ± 0.07	5.5 ± 0.3	

Appendix XVI

Subject details (normal controls) in the forearm blood flow study

Patient no.	Age (years)	Sex	Amount of alcohol/ week	Ascites	Enceph	Bili (umol/L)	ALT (iu/L)	GGT (iu/L)	Albumin (g/L)	PTR ratio	Childs Pugh Score	Smoker ?
P-1	62	M	No alcohol	N/A	N/A	13	51	13	39	N/A	N/A	No
P-2	54	M	6 cans beer/week	N/A	N/A	12	20	19	-	N/A	N/A	No
P-3	58	M	1/2 bottle wine/week	N/A	N/A	10	20	14	-	N/A	N/A	Yes
P-4	61	M	2 pints beer/week	N/A	N/A	3	18	14	43	N/A	N/A	Yes
P-5	47	M	No alcohol	N/A	N/A	8	31	47	40	N/A	N/A	No
P-6	54	M	3-4 pints beer/week	N/A	N/A	8	23	38	38	N/A	N/A	Yes
	56 ± 2.3	6M				9 ± 1.46	27.2 ± 5.1	24.2 ± 6.0	40 ± 1.1 (N=4)			

* The full blood count on each of the controls was normal. No control patient had a macrocytosis.

CONTROLS (n=6)

Forearm blood flow

CONTROL ARM

NAC dose (mg/min)	0	1	3	10	30	100	300
	4.91	4.67	4.68	4.39	4.29	4.8	4.36
	3.8	5.7	5.5	4.4	3.57	4.16	4.43
	2	2.36	1.58	0.45	0.18	0.64	0.76
	4.17	3.66	3.38	4.12	3.89	3.69	3.76
	2.52	1.54	1.39	1.54	1.39	1.43	1.86
	4.08	3.55	3.93	1.6	1.44	1.48	1.93
Mean blood flow	3.58	3.58	3.41	2.75	2.46	2.7	2.85
± SEM	0.45	0.65	0.67	0.72	0.69	0.7	0.63
	p > 0.1	p > 0.5	p > 0.5	p > 0.1	p < 0.05	p < 0.05	p < 0.01

Statistical comparison between flow in infused and control arm at each dose (paired t-test)

INFUSED ARM

NAC dose (mg/min)	0	1	3	10	30	100	300
	2.89	4.21	4.58	3.52	4.21	11.39	13.64
	4.09	4.20	4.38	4.40	4.60	6.65	10.63
	2.23	2.51	1.56	0.37	0.46	0.72	1.26
	5.46	5.16	4.58	5.35	5.02	6.22	9.94
	5.88	3.52	3.32	3.33	3.40	5.05	6.57
	4.47	3.38	3.59	3.35	3.42	5.09	6.61
Mean blood flow	4.17	3.83	3.67	3.39	3.52	5.85	8.81
± SEM	0.58	0.37	0.47	0.68	0.66	1.4	1.75

Appendix XVII

Forearm blood flow response to N-acetylcysteine - control group
(ml/dL forearm/minute)

CIRRHOTIC PATIENTS (n=6)

CONTROL ARM

NAC dose (mg/min)	0	1	3	10	30	100	300
	2.32	2.00	2.48	2.43	2.56	2.97	3.14
	2.69	2.59	2.42	2.57	3.37	3.77	3.45
	2.82	3.12	3.15	3.06	3.08	3.00	4.68
	3.63	2.85	2.78	2.62	2.68	2.64	3.25
	2.75	2.72	2.60	1.99	2.33	2.39	2.17
	3.88	3.9	3.51	2.64	2.10	1.92	2.13
Mean blood flow	3.02	2.86	2.82	2.55	2.69	2.78	3.14
± SEM	0.25	0.26	0.17	0.14	0.19	0.26	0.38
	p >> 0.5	p > 0.5	p > 0.5	p > 0.1	p > 0.1	p < 0.05	p < 0.01

Statistical comparison between flow in infused and control arm at each dose (paired t-test)

INFUSED ARM

NAC dose (mg/min)	0	1	3	10	30	100	300
	2.19	2.28	2.49	2.64	2.91	5.15	9.83
	3.07	3.27	3.15	7.01	6.42	12.38	26.7
	3.68	4.06	4.37	4.9	6.06	7.98	13.14
	3.0	2.53	2.7	2.83	2.46	3.50	8.41
	3.11	2.99	2.72	2.79	2.51	4.63	8.54
	2.97	2.74	2.61	1.94	1.70	3.77	9.70
Mean blood flow	3.00	3.00	3.00	3.69	3.68	6.24	12.72
± SEM	0.19	0.26	0.29	0.78	0.83	1.39	2.88

Appendix XVIII

Forearm blood flow response to N-acetylcysteine in patients with cirrhosis
(ml/dL forearm/minute)

CONTROL GROUP (n=6)

NAC dose (mg/min)	0	1	3	10	30	100	300
	0.83	0.93	0.92	1.0	-7.36	1.69	2.85
	1.33	1.42	1.36	1.32	1.30	1.69	2.68
	2.42	2.33	2.43	2.19	2.47	3.54	3.53
	1.09	0.97	0.92	2.12	2.39	3.43	3.41
	1.11	1.11	0.74	1.01	10.7	1.4	2.62
	0.78	0.74	0.83	0.82	1.18	2.71	3.09
Mean ratio of infused/control arms	1.26	1.25	1.20	1.41	1.78	2.41	3.03
± SEM	0.25	0.24	0.26	0.24	0.27	0.39	0.15
	p > 0.1	p > 0.1	p > 0.5	p > 0.5	p > 0.1	p > 0.5	p > 0.1

Statistical comparison between ratios in cirrhotic patients and controls (unpaired t-test)

CIRRHOTIC PATIENTS (N=6)

NAC dose (mg/min)	0	1	3	10	30	100	300
	0.77	0.71	0.75	1.74	0.82	1.97	5.4
	1.14	1.15	1.08	1.43	1.11	2.15	4.12
	0.85	0.89	0.98	1.08	0.92	1.33	2.61
	1.32	1.32	1.40	1.62	1.98	2.71	2.83
	1.15	1.29	1.31	1.38	1.91	3.33	8.23
	0.95	1.16	0.99	1.08	1.14	1.76	3.41
Mean ratio of infused/control arms	1.03	1.09	1.09	1.22	1.31	2.21	4.43
± SEM	0.08	0.1	0.1	0.13	0.2	0.30	0.86

Appendix XIX

Forearm blood flow ratios (infused / control arm) in response to NAC in cirrhotic patients compared with controls.

CONTROL GROUP (n=6)

NAC dose (mg/min)	1	3	10	30	100	300
	4.6.	0.27	-3	-4.7	24.3	97.2
	8.0	12.7	1.8	14.5	64.6	63.7
	-2	-7	115.5	142.4	248.5	246.6
	-9.1	-39.4	-17.4	84.9	38	134.6
	2.0	6.6	14.1	21.0	58.9	198.2
	5.0	7.1	4.8	52.2	249	298
Mean % change in forearm flow in response to NAC	1.42	-3.29	19.3	51.72	113.9	173
± SEM	2.5	7.7	19.7	22.3	43.1	36.8
	p > 0.5	p > 0.5	p > 0.5	p > 0.1	p >> 0.5	p > 0.1

Statistical comparison between % change in flow to NAC between cirrhotic patients and controls (unpaired t-test)

CIRRHOTIC PATIENTS (N=6)

NAC dose (mg/min)	1	3	10	30	100	300
	-16.8	-11.4	-12.9	-3.1	132.8	539
	1.1	-5.9	24.5	-2.8	88.5	261.0
	-7.3	1.82	12.8	-4.1	38.0	172.5
	11.4	17.7	36.3	67.2	128.0	138.6
	16.21	18.1	24.6	72.4	199.8	641.6
	14.8	-2	7.55	13.3	74.6	233.7
Mean % change in forearm flow in response to NAC	3.24	3.05	15.5	23.82	110.2	331.9
± SEM	5.43	5.02	7.01	14.8	22.9	84.7

Appendix XX

Percentage change in forearm flow from baseline in response to NAC in cirrhotic patients compared with controls.

Appendix XXI **Cirrhotic patients details - pharmacokinetic study**

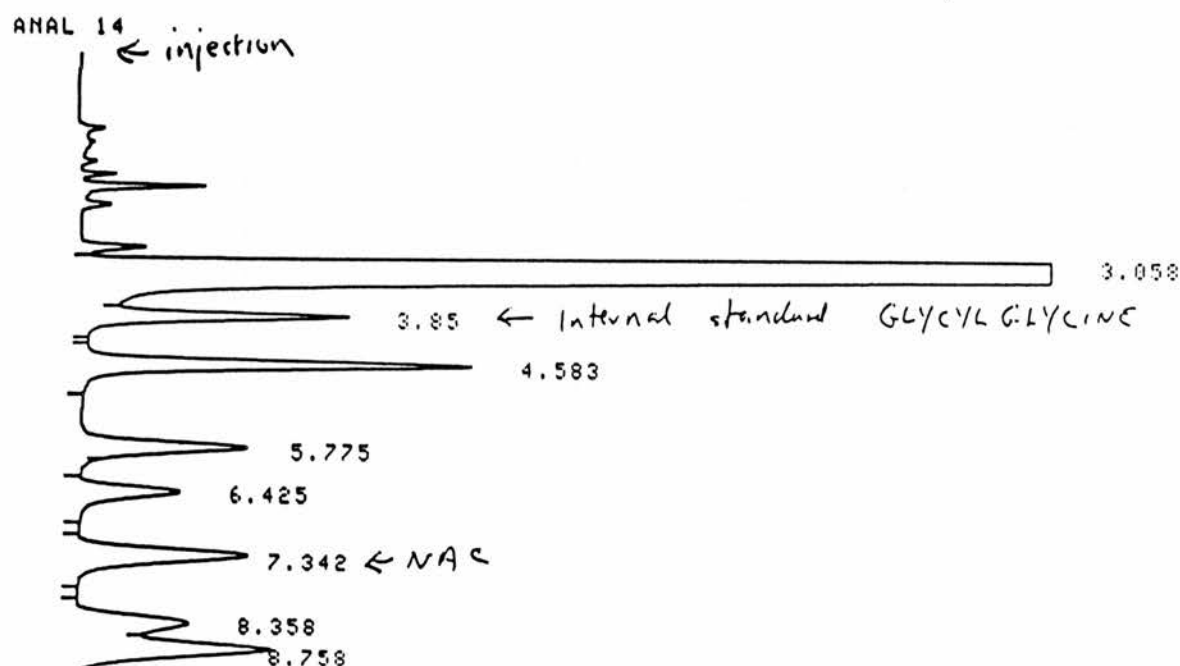
Patient no.	Age (years)	Sex	Aetiol	Ascites	Enceph	Bili (umol/L)	ALT (iu/L)	GGT (iu/L)	Alb (g/L)	PTR ratio	Childs Pugh Score
P-1	54*	M	ALC	Marked	No	48	36	162	33	14/12=1.17	B(9)
P-2	49*	F	ALC	Marked	No	202	60	359	43	23/12=1.92	C(11)
P-3	49	M	PBC	Slight	No	15	20	146	42	12/12=1.0	A(5)
P-4	49	M	2BC	Slight	No	103	51	242	31	14/14=1.00	B(7)
P-5	74	M	ALC	No	No	36	57	216	33	12/12=1.00	B(7)
P-6	43	M	ALC	Slight	No	92	50	33	25	18/12=1.5	C(11)
P-7	40*	F	ALC	Moderate	Grade 1	27	12	436	22	15/12=1.25	C(10)
P-8	66*	M	ALC	Moderate	Grade 2	125	25	66	27	17/12=1.42	C(13)
P-9	58	M	ALC	No	No	38	13	70	29	14/12=1.17	B(7)
51.1 ± 11.1											

* = on spironolactone

Appendix XXII

Sample HPLC trace showing glycylglycine and N-acetylcysteine peaks eluted.

421202



PKNO	TIME	RRT	HIGHT	HK	IDNO	CONC	NAME
1	3.058	0.7944	1097644	E			
2	3.85	1	36267	V	1		INT STD
3	4.583	1.1905	53521				
4	5.775	1.5	11029				
5	6.425	1.6688	14008				
6	7.342	1.9069	23540		2	23.8081	NAC
7	8.358	2.171	15878				
8	8.758	2.2749	27471	V			
TOTAL			1279357			23.8081	

Appendix XXIII

Estimates of plasma N-acetylcysteine at the time points for each subject

Time (h)	NAC F mg/L C1	Time (h)	NAC M mg/L C2	Time (h)	NAC M mg/L C3	Time (h)	NAC F mg/L C4	Time (h)	NAC M mg/L C5	Time (h)	NAC M mg/L C6
88 kg		77 kg		85 kg		53 kg		58 kg		74 kg	
0	0	0	0	0	0	0	0	0	0	0	0
0.33	45.7	0.33	40.6	0.33	40.7	0.33	109	0.33	65.2	0.17	60.2
0.67	27.7	0.67	25.5	0.67	24	0.67	31.5	0.67	40.1	0.33	36.2
1.25	16.8	1.17	14.9	1	18.9	1	28.6	1	27.8	0.67	25.3
1.5	10.8	1.5	12.4	1.5	14.7	1.5	16.9	1.5	20.9	1	19.5
2	8.3	2	9.4	2	10.4	2	12.8	2	13.7	2	13.2
3	5.2	3	9	3	7.7	3	7.2	3	8.2	3	9.3
4	4.1	4	6.4	4	4.6	4	5.2	4	10.2	4	6.4
5	3.2	6	4	6	3	6	3.4	6	5	6	3.8
6	2.6	8	2.8	8	2.4	8	2	8	2.5	8	2.9
7	1.9	10	2.2	10	1.8			10	1.8		
10	0.6							12	1.4		
12	0.6										

weight -->

Key: C = Control (healthy volunteer) (n = 7)
P = Patient (cirrhosis) (n = 9)
Weight: to the nearest kg

contd/

Appendix XXIII, contd

Time (h)	NAC	Time (h)	NAC	Time (h)	NAC	Time (h)	NAC	Time (h)	NAC	Time (h)	NAC	Time (h)	NAC	Time (h)	NAC
M	mg/L	F	mg/L	M	mg/L	F	mg/L	M	mg/L	M	mg/L	M	mg/L	M	mg/L
P1		P2		P3		P4		P5		P6		P7		P8	
87 kg		65 kg		72 kg		51 kg		66 kg		73 kg		56 kg		66 kg	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.33	28.1	0.33	585.2	0.33	41.7	0.33	86.2	0.17	47	0.33	41.6	0.17	37.3	0.17	85
0.67	23.3	0.67	34.9	0.67	34.7	0.67	55.9	0.33	40.8	0.75	24.5	0.33	23.5	0.33	31.3
1	22.4	1	27.8	1	28.6	1	49.3	0.67	26.8	1	21.8	0.75	19.2	0.67	27.5
1.5	18.2	1.5	20.6	2	21.3	1.5	39.8	1	22.3	1.5	17.6	1	14.6	1	23.2
2	12.2	2	22.2	3	16.5	2	31.9	1.5	16.8	2	14.7	1.5	13.7	1.5	18.4
3	10	4	14.4	4	12.8	3	31.2	2	13.2	3	14.1	2	10.7	2	16.6
4	7.7	6	7.1	6	8.8	4	27.7	3	12.6	4	13	3	9.4	3	11.6
6.5	4	10	5.6	8	6.5	6	17.9	4	9.4	6	9.5	4	6.6	4	10.2
8	3.5			10	5.3	8	12.1	6	6.6	8	7.6	6	5.2	6	6.8
10	2.4					10	10.9	8	5.5	10	6.1	8	3.4	8	5.8

weight
--->

Key: C = Control (healthy volunteer) (n = 7)
P = Patient (cirrhosis) (n = 9)
Weight: to the nearest kg

Appendix XXIV

Each subjects pharmacokinetic analysis

SIPHAR/PC : Version 4.0

Date: 12-14-1993

Time: 10:17:2

Title : NAC

Data set : C-1

The minimization algorithm used is : POWELL

The structural model is :

Two exponential(s), after I.V. bolus

The model has been fitted to data using weighted least squares algorithm
with the weighting factor = $1/y(\text{calc})^2$

Parameters		Half-life (h)
Coef(1) (mg/l)	: 9.8780	
Exp (1) (1/h)	: 0.2291	3.0260
Coef(2) (mg/l)	: 63.7039	
Exp (2) (1/h)	: 1.6984	0.4081

Dose admin. (mg) : 600.00

A.U.C. (h *mg/l) (trapezoidal rule) :

between	0.0000 and	0.3300	: 19.6815
between	0.3300 and	7.0000	: 53.8080
between	7.0000 and	infinity	: 8.2948

Total AUC : 81.7843

% of total AUC extrapolated : 34.2074

MRT (Trapezoidal rule) : 2.5109

Total clearance/F (*) : 7.3364

Volume of distribution/F (beta phase) (+) : 32.0281

Parameters computed using the fitted model :

Vdss (Volume at steady state)/F	:	19.4125
AUC (0 - infinity)	:	80.6313
MRT (Mean residence time)	:	2.6088

(*) Clearance unit = dose units / AUC units

(+) Volume unit = dose units / concentrations units

Total clearance and Volumes are computed assuming F=1

SIPHAR/PC : Version 4.0

Date: 12-14-1993

Title : NAC

Time: 10:18:3

Data set : C-2

The minimization algorithm used is : POWELL

The structural model is :

Two exponential(s), after I.V. bolus

The model has been fitted to data using weighted least squares algorithm
with the weighting factor = $1/y(\text{calc})^2$

Parameters		Half-life (h)
Coef(1) (mg/l)	: 14.1102	
Exp (1) (1/h)	: 0.1930	3.5908
Coef(2) (mg/l)	: 61.0256	
Exp (2) (1/h)	: 2.3703	0.2924

Dose admin. (mg) : 600.00

A.U.C. (h *mg/l) (trapezoidal rule) :

between	0.0000 and	0.3300	:	19.0964
between	0.3300 and	10.0000	:	70.3915
between	10.0000 and	infinity	:	11.3970

Total AUC	:	100.8849
% of total AUC extrapolated	:	30.2259

MRT (Trapezoidal rule) : 3.9047

Total clearance/F (*) : 5.9474

Volume of distribution/F (beta phase) (+) : 30.8100

Parameters computed using the fitted model :

Vdss (Volume at steady state)/F	:	23.9226
AUC (0 - infinity)	:	98.8429
MRT (Mean residence time)	:	3.9410

(*) Clearance unit = dose units / AUC units

(+) Volume unit = dose units / concentrations units

Total clearance and Volumes are computed assuming F=1

SIPHAR/PC : Version 4.0

Date: 12-14-1993
Title : NAC

Time: 10:20:0
Data set : C-3

The minimization algorithm used is : POWELL

The structural model is :

Two exponential(s), after I.V. bolus

The model has been fitted to data using weighted least squares algorithm
with the weighting factor = $1/y(\text{calc})^2$

Parameters		Half-life (h)
Coef(1) (mg/l)	: 7.7808	
Exp (1) (1/h)	: 0.1486	4.6631
Coef(2) (mg/l)	: 40.9172	
Exp (2) (1/h)	: 1.0571	0.6557

Dose admin. (mg) : 600.00
A.U.C. (h *mg/l) (trapezoidal rule) :
between 0.0000 and 0.3300 : 14.7507
between 0.3300 and 10.0000 : 65.1525
between 10.0000 and infinity : 12.1093

Total AUC : 92.0125
% of total AUC extrapolated : 29.1917

MRT (Trapezoidal rule) : 4.2387
Total clearance/F (*) : 6.5209
Volume of distribution/F (beta phase) (+) : 43.8684

Parameters computed using the fitted model :

Vdss (Volume at steady state)/F : 28.1351
AUC (0 - infinity) : 91.0529
MRT (Mean residence time) : 4.2696

(*) Clearance unit = dose units / AUC units
(+) Volume unit = dose units / concentrations units
Total clearance and Volumes are computed assuming F=1

SIPHAR/PC : Version 4.0

Date: 12-14-1993
Title : NAC

Time: 10:20:5
Data set : C-4

The minimization algorithm used is : POWELL

The structural model is :

Two exponential(s), after I.V. bolus

The model has been fitted to data using weighted least squares algorithm
with the weighting factor = $1/y(\text{calc})^2$

Parameters		Half-life (h)
Coef(1) (mg/l)	: 18.9760	
Exp (1) (1/h)	: 0.2873	2.4129
Coef(2) (mg/l)	: 199.8582	
Exp (2) (1/h)	: 2.7328	0.2536

Dose admin. (mg) : 600.00
A.U.C. (h *mg/l) (trapezoidal rule) :
between 0.0000 and 0.3300 : 54.0926
between 0.3300 and 8.0000 : 82.8015
between 8.0000 and infinity : 6.9621

Total AUC : 143.8562
% of total AUC extrapolated : 42.4415

MRT (Trapezoidal rule) : 1.7545
Total clearance/F (*) : 4.1708
Volume of distribution/F (beta phase) (+) : 14.5188

Parameters computed using the fitted model :

Vdss (Volume at steady state)/F	:	7.9500
AUC (0 - infinity)	:	139.1902
MRT (Mean residence time)	:	1.8443

(*) Clearance unit = dose units / AUC units

(+) Volume unit = dose units / concentrations units

Total clearance and Volumes are computed assuming F=1

SIPHAR/PC : Version 4.0

Date: 12-14-1993

Time: 10:25:2

Title :

Data set : C-5

The minimization algorithm used is : POWELL

The structural model is :

Two exponential(s), after I.V. bolus

The model has been fitted to data using weighted least squares algorithm
with the weighting factor = $1/y(\text{calc})^2$

Parameters		Half-life (h)
Coef(1) (mg/l)	: 19.1485	
Exp (1) (1/h)	: 0.2263	3.0629
Coef(2) (mg/l)	: 51.5092	
Exp (2) (1/h)	: 1.3174	0.5261

Dose admin. (mg) : 600.00
A.U.C. (h *mg/l) (trapezoidal rule) :
between 0.0000 and 0.3300 : 22.4165
between 0.3300 and 12.0000 : 87.7505
between 12.0000 and infinity : 6.1864

Total AUC : 116.3535
% of total AUC extrapolated : 24.5828

MRT (Trapezoidal rule) : 3.4142
Total clearance/F (*) : 5.1567
Volume of distribution/F (beta phase) (+) : 22.7868

Parameters computed using the fitted model :

Vdss (Volume at steady state)/F	:	15.8215
AUC (0 - infinity)	:	123.7137
MRT (Mean residence time)	:	3.2622

(*) Clearance unit = dose units / AUC units
(+) Volume unit = dose units / concentrations units
Total clearance and Volumes are computed assuming F=1

SIPHAR/PC : Version 4.0

Date: 12-14-1993

Time: 10:27:4

Title :

Data set : C- 6

The minimization algorithm used is : POWELL

The structural model is :

Two exponential(s), after I.V. bolus

The model has been fitted to data using weighted least squares algorithm
with the weighting factor = $1/y(\text{calc})^2$

Parameters		Half-life (h)
Coef(1) (mg/l)	: 19.9982	
Exp (1) (1/h)	: 0.2536	2.7337
Coef(2) (mg/l)	: 61.6822	
Exp (2) (1/h)	: 2.9925	0.2316

Dose admin. (mg) : 600.00

A.U.C. (h *mg/l) (trapezoidal rule) :

between 0.0000 and 0.1700 : 12.0598

between 0.1700 and 8.0000 : 77.9090

between 8.0000 and infinity : 11.4371

Total AUC : 101.4059

% of total AUC extrapolated : 23.1712

MRT (Trapezoidal rule) : 3.1697

Total clearance/F (*) : 5.9168

Volume of distribution/F (beta phase) (+) : 23.3349

Parameters computed using the fitted model :

Vdss (Volume at steady state)/F	:	19.2753
AUC (0 - infinity)	:	99.4821
MRT (Mean residence time)	:	3.1959

(*) Clearance unit = dose units / AUC units

(+) Volume unit = dose units / concentrations units

Total clearance and Volumes are computed assuming F=1

SIPHAR/PC : Version 4.0

Date: 12-14-1993

Time: 10:28:4

Title :

Data set : P-J

The minimization algorithm used is : POWELL

The structural model is :

Two exponential(s), after I.V. bolus

The model has been fitted to data using weighted least squares algorithm
with the weighting factor = $1/y(\text{calc})^2$

Parameters		Half-life (h)
Coef(1) (mg/l)	: 9.3748	
Exp (1) (1/h)	: 0.1364	5.0817
Coef(2) (mg/l)	: 23.9649	
Exp (2) (1/h)	: 0.6224	1.1137

Dose admin. (mg) : 600.00

A.U.C. (h *mg/l) (trapezoidal rule) :

between	0.0000	and	0.3300	:	10.1375
between	0.3300	and	10.0000	:	80.1285
between	10.0000	and	infinity	:	17.5954

Total AUC : 107.8614

% of total AUC extrapolated : 25.7116

MRT (Trapezoidal rule) : 5.2237

Total clearance/F (*) : 5.5627

Volume of distribution/F (beta phase) (+) : 40.7824

Parameters computed using the fitted model :

Vdss (Volume at steady state)/F	:	29.5192
AUC (0 - infinity)	:	107.2357
MRT (Mean residence time)	:	5.2759

(*) Clearance unit = dose units / AUC units

(+) Volume unit = dose units / concentrations units

Total clearance and Volumes are computed assuming F=1

SIPHAR/PC : Version 4.0

Date: 12-14-1993

Time: 10:34:0

Title :

Data set : P-2

The minimization algorithm used is : POWELL

The structural model is :

Two exponential(s), after I.V. bolus

The model has been fitted to data using weighted least squares algorithm
with the weighting factor = $1/y(\text{calc})^2$

Parameters		Half-life (h)
Coef(1) (mg/l)	: 34.6940	
Exp (1) (1/h)	: 0.2488	2.7865
Coef(2) (mg/l)	: 722.6889	
Exp (2) (1/h)	: 7.2678	0.0954

Dose admin. (mg) : 600.00
A.U.C. (h *mg/l) (trapezoidal rule) :
between 0.0000 and 0.6700 : 265.4148
between 0.6700 and 6.0000 : 91.2455
between 6.0000 and infinity : 28.5422

Total AUC : 385.2025
% of total AUC extrapolated : 76.3123

MRT (Trapezoidal rule) : 1.3966
Total clearance/F (*) : 1.5576
Volume of distribution/F (beta phase) (+) : 6.2617

Parameters computed using the fitted model :

Vdss (Volume at steady state)/F : 6.0377
AUC (0 - infinity) : 238.9081
MRT (Mean residence time) : 2.4041

- (*) Clearance unit = dose units / AUC units
(+) Volume unit = dose units / concentrations units
Total clearance and Volumes are computed assuming F=1

SIPHAR/PC : Version 4.0

Date: 12-14-1993

Time: 10:35:0

Title :

Data set : P-3

The minimization algorithm used is : POWELL

The structural model is :

Two exponential(s), after I.V. bolus

The model has been fitted to data using weighted least squares algorithm
with the weighting factor = $1/y(\text{calc})^2$

Parameters		Half-life (h)
Coef(1) (mg/l)	: 16.6745	
Exp (1) (1/h)	: 0.1173	5.9105
Coef(2) (mg/l)	: 29.9241	
Exp (2) (1/h)	: 0.6483	1.0692

Dose admin. (mg) : 600.00
A.U.C. (h *mg/l) (trapezoidal rule) :
between 0.0000 and 0.3300 : 14.5693
between 0.3300 and 10.0000 : 130.6325
between 10.0000 and infinity : 45.1935

Total AUC : 190.3953
% of total AUC extrapolated : 31.3888

MRT (Trapezoidal rule) : 6.8291
Total clearance/F (*) : 3.1513
Volume of distribution/F (beta phase) (+) : 26.8717

Parameters computed using the fitted model :

Vdss (Volume at steady state)/F	:	21.7116
AUC (0 - infinity)	:	188.3420
MRT (Mean residence time)	:	6.8154

(*) Clearance unit = dose units / AUC units
(+) Volume unit = dose units / concentrations units
Total clearance and Volumes are computed assuming F=1

SIPHAR/PC : Version 4.0

Date: 12-14-1993

Time: 10:36:0

Title :

Data set : P-4

The minimization algorithm used is : POWELL

The structural model is :

Two exponential(s), after I.V. bolus

The model has been fitted to data using weighted least squares algorithm
with the weighting factor = $1/y(\text{calc})^2$

Parameters		Half-life (h)
Coef(1) (mg/l)	: 47.6307	
Exp (1) (1/h)	: 0.1547	4.4805
Coef(2) (mg/l)	: 101.0034	
Exp (2) (1/h)	: 2.7977	0.2478

Dose admin. (mg) : 600.00
A.U.C. (h *mg/l) (trapezoidal rule) :
between 0.0000 and 0.3300 : 38.7476
between 0.3300 and 10.0000 : 241.3150
between 10.0000 and infinity : 70.4575

Total AUC : 350.5202
% of total AUC extrapolated : 31.1552

MRT (Trapezoidal rule) : 5.8697
Total clearance/F (*) : 1.7117
Volume of distribution/F (beta phase) (+) : 11.0647

Parameters computed using the fitted model :

Vdss (Volume at steady state)/F	:	10.1570
AUC (0 - infinity)	:	343.9866
MRT (Mean residence time)	:	5.8231

- (*) Clearance unit = dose units / AUC units
(+) Volume unit = dose units / concentrations units
Total clearance and Volumes are computed assuming F=1

SIPHAR/PC : Version 4.0

Date: 12-14-1993

Time: 10:36:5

Title :

Data set : P-5

The minimization algorithm used is : POWELL

The structural model is :

Two exponential(s), after I.V. bolus

The model has been fitted to data using weighted least squares algorithm
with the weighting factor = $1/y(\text{calc})^2$

Parameters		Half-life (h)
Coef(1) (mg/l)	: 17.9438	
Exp (1) (1/h)	: 0.1530	4.5304
Coef(2) (mg/l)	: 41.9979	
Exp (2) (1/h)	: 1.9054	0.3638

Dose admin. (mg) : 600.00

A.U.C. (h *mg/l) (trapezoidal rule) :

between	0.0000 and	0.1700	: 9.0900
between	0.1700 and	8.0000	: 95.8925
between	8.0000 and	infinity	: 35.9479

Total AUC : 140.9304

% of total AUC extrapolated : 31.9576

MRT (Trapezoidal rule) : 5.6219

Total clearance/F (*) : 4.2574

Volume of distribution/F (beta phase) (+) : 27.8264

Parameters computed using the fitted model :

Vdss (Volume at steady state)/F	:	24.0521
AUC (0 - infinity)	:	139.3220
MRT (Mean residence time)	:	5.5850

(*) Clearance unit = dose units / AUC units

(+) Volume unit = dose units / concentrations units

Total clearance and Volumes are computed assuming F=1

SIPHAR/PC : Version 4.0

Date: 12-14-1993

Time: 10:37:3

Title :

Data set : P-6

The minimization algorithm used is : POWELL

The structural model is :

Two exponential(s), after I.V. bolus

The model has been fitted to data using weighted least squares algorithm
with the weighting factor = $1/y(\text{calc})^2$

Parameters		Half-life (h)
Coef(1) (mg/l)	: 19.7529	
Exp (1) (1/h)	: 0.1180	5.8745
Coef(2) (mg/l)	: 55.8024	
Exp (2) (1/h)	: 2.7559	0.2515

Dose admin. (mg) : 600.00
A.U.C. (h *mg/l) (trapezoidal rule) :
between 0.0000 and 0.3300 : 19.3306
between 0.3300 and 10.0000 : 118.8435
between 10.0000 and infinity : 51.6978

Total AUC : 189.8720
% of total AUC extrapolated : 37.4086

MRT (Trapezoidal rule) : 7.5143
Total clearance/F (*) : 3.1600
Volume of distribution/F (beta phase) (+) : 26.7814

Parameters computed using the fitted model :

Vdss (Volume at steady state)/F : 24.2990
AUC (0 - infinity) : 187.6555
MRT (Mean residence time) : 7.5997

(*) Clearance unit = dose units / AUC units
(+) Volume unit = dose units / concentrations units
Total clearance and Volumes are computed assuming F=1

SIPHAR/PC : Version 4.0

Date: 12-14-1993

Time: 10:38:1

Title :

Data set : P-7

The minimization algorithm used is : POWELL

The structural model is :

Two exponential(s), after I.V. bolus

The model has been fitted to data using weighted least squares algorithm
with the weighting factor = $1/y(\text{calc})^2$

Parameters		Half-life (h)
Coef(1) (mg/l)	: 16.6456	
Exp (1) (1/h)	: 0.1998	3.4694
Coef(2) (mg/l)	: 31.0225	
Exp (2) (1/h)	: 2.9261	0.2369

Dose admin. (mg) : 600.00
A.U.C. (h *mg/l) (trapezoidal rule) :
 between 0.0000 and 0.1700 : 7.2223
 between 0.1700 and 8.0000 : 69.6810
 between 8.0000 and infinity : 17.0179

 Total AUC : 93.9212
 % of total AUC extrapolated : 25.8090

MRT (Trapezoidal rule) : 4.4574
Total clearance/F (*) : 6.3883
Volume of distribution/F (beta phase) (+) : 31.9753

Parameters computed using the fitted model :

Vdss (Volume at steady state)/F	:	28.6132
AUC (0 - infinity)	:	93.9177
MRT (Mean residence time)	:	4.4788

(*) Clearance unit = dose units / AUC units

(+) Volume unit = dose units / concentrations units

Total clearance and Volumes are computed assuming F=1

SIPHAR/PC : Version 4.0

Date: 12-14-1993

Time: 10:39:2

Title :

Data set : P-8

The minimization algorithm used is : POWELL

The structural model is :

Two exponential(s), after I.V. bolus

The model has been fitted to data using weighted least squares algorithm
with the weighting factor = $1/y(\text{calc})^2$

Parameters		Half-life (h)
Coef(1) (mg/l)	: 26.7062	
Exp (1) (1/h)	: 0.2089	3.3188
Coef(2) (mg/l)	: 623.2614	
Exp (2) (1/h)	: 13.8583	0.0500

Dose admin. (mg) : 600.00
A.U.C. (h *mg/l) (trapezoidal rule) :
between 0.0000 and 0.1700 : 62.4723
between 0.1700 and 8.0000 : 101.3655
between 8.0000 and infinity : 27.7702

Total AUC : 191.6080
% of total AUC extrapolated : 47.0975

MRT (Trapezoidal rule) : 3.3327
Total clearance/F (*) : 3.1314
Volume of distribution/F (beta phase) (+) : 14.9930

Parameters computed using the fitted model :

Vdss (Volume at steady state)/F	:	12.3612
AUC (0 - infinity)	:	172.8423
MRT (Mean residence time)	:	3.5609

(*) Clearance unit = dose units / AUC units

(+) Volume unit = dose units / concentrations units

Total clearance and Volumes are computed assuming F=1

SIPHAR/PC : Version 4.0

Date: 12-14-1993

Time: 10:40:26

Title :

Data set : P-9

The minimization algorithm used is : POWELL

The structural model is :

Two exponential(s), after I.V. bolus

The model has been fitted to data using weighted least squares algorithm
with the weighting factor = $1/y(\text{calc})^2$

Parameters		Half-life (h)
Coef(1) (mg/l)	: 19.3098	
Exp (1) (1/h)	: 0.2473	2.8030
Coef(2) (mg/l)	: 453.0135	
Exp (2) (1/h)	: 13.1127	0.0529

Dose admin. (mg) : 600.00
A.U.C. (h *mg/l) (trapezoidal rule) :
between 0.0000 and 0.1700 : 45.8765
between 0.1700 and 8.0000 : 69.6590
between 8.0000 and infinity : 10.5141

Total AUC : 126.0496
% of total AUC extrapolated : 44.7368

MRT (Trapezoidal rule) : 2.4856
Total clearance/F (*) : 4.7600
Volume of distribution/F (beta phase) (+) : 19.2491

Parameters computed using the fitted model :

Vdss (Volume at steady state)/F	:	15.0590
AUC (0 - infinity)	:	112.6342
MRT (Mean residence time)	:	2.8269

(*) Clearance unit = dose units / AUC units

(+) Volume unit = dose units / concentrations units

Total clearance and Volumes are computed assuming F=1

Key to Appendix XXV

A = elimination half-life (hours)

B = total clearance (L/h)

C = mean residence time (h)

D = area under the curve mg/L x h

E = volume of distribution at study state

F = bilirubin ($\mu\text{mol/L}$)

G = ALT (iu/L)

H = albumin (g/L)

I = PTR ratio

J = age (years)

Appendix XXV

Correlation of Childs' Pugh Variables and pharmacokinetic parameters - patients with liver cirrhosis

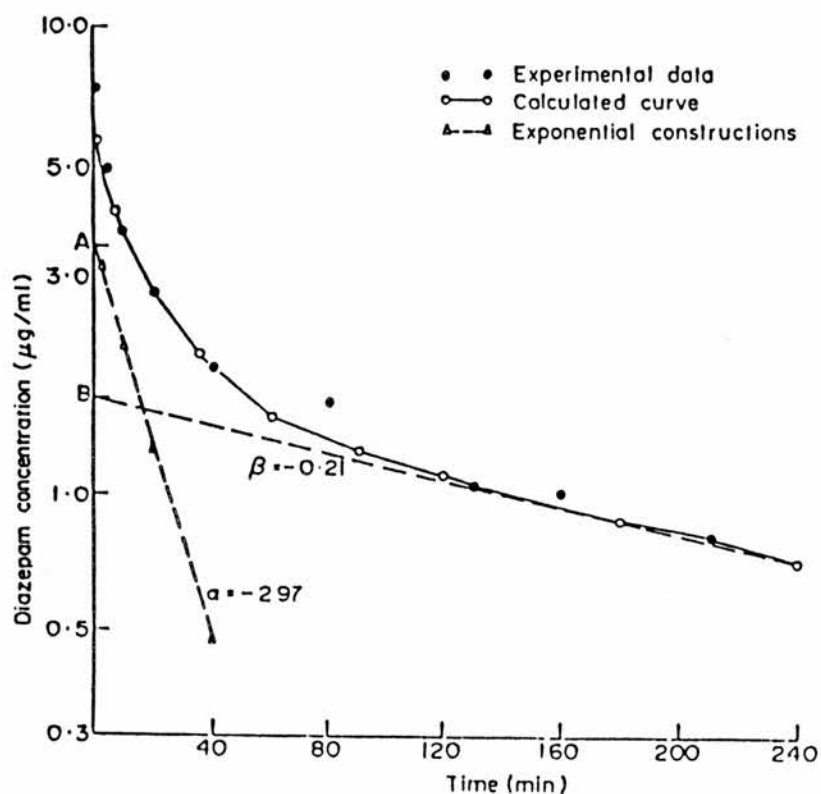
MATRIX OF SPEARMAN CORRELATION COEFFICIENTS

A	B	C	D	E
1.000	1.000	1.000	1.000	1.000
0.217	0.033	-0.033	-0.883	-0.650
0.917	1.000	0.333	0.667	-0.367
-0.217	0.983	-0.667	0.617	-0.285
0.550	-0.667	0.067	0.452	-0.213
-0.517	-0.617	-0.109	0.162	-0.068
-0.050	-0.452	-0.434	0.051	
0.042	-0.162	-0.288		
-0.451	-0.051			
-0.186				

F	G	H	I	J
1.000	1.000	1.000	1.000	1.000
0.583	0.544	-0.252	-0.320	
0.084	0.587	0.298		
0.587	0.203			
0.102				

NUMBER OF OBSERVATIONS: 9

Patient no.	A	B	C	D	E	F	G	H	I	J
P-1	5.08	5.56	5.22	107.86	29.06	48	36	33	1.17	54
P-2	2.79	1.56	1.4	385.2	2.18	202	60	43	1.92	49
P-3	5.91	3.15	6.83	190.4	21.52	15	20	42	1	49
P-4	4.48	1.71	5.87	350.52	10.05	103	51	31	1	49
P-5	4.53	4.26	5.62	140.93	23.93	36	57	33	1	74
P-6	5.87	3.16	7.51	189.87	23.75	92	50	25	1.5	43
P-7	3.47	6.39	4.46	93.92	28.48	27	12	22	1.25	40
P-8	3.32	3.13	3.33	191.61	10.44	125	25	27	1.42	66
P-9	2.8	4.76	2.49	126.05	11.83	38	13	29	1.17	58



Appendix XXVI

Experimental data, fitting a calculated curve and determining the equation for a pharmacokinetic study.

$$(pt = 3.45 \exp (-2.97t) + 1.65 \exp (-0.21t))$$

Appendix XXVII

Publications to date

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Clinical review

Organic Nitrates in Portal Hypertension

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INTRODUCTION

Variceal hemorrhage is a major cause of morbidity and mortality in cirrhosis. Pharmacological therapy of portal hypertension aims to arrest acute variceal bleeding, prevent rebleeding or to provide prophylaxis for patients who have not bled. The purpose of this review is to discuss the pharmacology and hemodynamic action of organic nitrates and their clinical use in portal hypertension.

PHARMACOLOGY OF ORGANIC NITRATES

Isosorbide dinitrate and isosorbide-5-mononitrate (Is-5-Mn) are "long-acting" organic nitrates. The mononitrate is the active component and is formed from dinitrates by rapid denitration in the liver (1-3). It is a powerful venous and mild arterial dilator with dose-dependent activity (4) and little first pass metabolism (5). Is-5-Mn has a prolonged half-life (5 h) (5, 6) and dose linear kinetics (7) even in the presence of liver disease (8, 9) or renal failure (10). In contrast, the dinitrate has a high first-pass effect with an apparent oral bioavailability of 0.2 in normal subjects (11). This increases up to a mean of 0.65 in cirrhosis because of reduced hepatic extraction and the presence of portasystemic shunts (12). As these show interindividual variation, the appropriate dosage of the dinitrate is difficult to determine and the response is unpredictable in patients with chronic liver disease (13). Such reasons make Is-5-Mn preferable to other organic nitrates in patients with cirrhosis.

Adverse reactions associated with nitrates are unusual although mild headache resolving within 24 h of the first dose occurs in up to one-third of patients (12).

The molecular mechanism of vasodilation by nitrates is controversial. In 1977 two groups demonstrated a dose-dependent increase in the levels of cyclic-guano-

sine 5'-monophosphate (GMP) in smooth muscle after nitrate administration (14, 15). Subsequently it was shown that many other vasodilators, including nitric oxide, activate soluble guanylate cyclase (16, 17). Some vasodilators have been shown to generate nitric oxide in a nonenzymic reaction with cysteine (18). However, nitrates do not directly stimulate nitric oxide synthetase as demonstrated by Vallance *et al.* (19) who showed that glyceryltrinitrate infusion at 2 and 5 nmol/min to human controls caused a dose-dependent increase in forearm blood flow which was unaltered by L-N-monomethyl-arginine administration (a specific inhibitor of nitric oxide synthetase). It therefore seems likely that nitrates produce an increase in cyclic-GMP levels in smooth muscle by direct action rather than via nitric oxide synthetase and there is evidence for thiol intermediates as modulators of these cellular events (20).

ACUTE EFFECTS OF ORGANIC NITRATES ON PORTAL AND SYSTEMIC HEMODYNAMICS

Animal studies

Vatner *et al.* (21) demonstrated that intravenous nitroglycerin administration to normal dogs resulted in a significant decrease in mesenteric blood flow after an initial increase. The fall in mesenteric blood flow was associated with a reduction in arterial pressure and an increase in heart rate and it was suggested that nitrate-induced venodilation resulted in baroreceptor-mediated mesenteric arterial vasoconstriction, a sympathetic response to vasodilation and venous pooling (a reduction in preload accounting for the reduced cardiac output).

In the portal vein-ligated rat (an experimental model of portal hypertension) splanchnic vasoconstriction has been demonstrated after nitrate administration (22-24).

Other studies have demonstrated that nitrates may reduce portal pressure by more than one mechanism,

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e.g., myofibroblasts have been demonstrated in a perisinusoidal location in the cirrhotic liver and may be involved in the increased resistance to portal flow (25). In the isolated perfused cirrhotic rat liver, nitrates decrease intrahepatic resistance (26). Nitrates may also have a direct vasodilatory effect on the collateral circulation; high-dose nitrates given to portal vein-ligated rats were shown to reduce resistance across collateral vessels (24).

Human studies

In humans, noninvasive methods to assess portal pressure, portal flow, and portal-systemic shunting are limited. However, an invasive balloon catheter technique allows measurement of the hepatic venous pressure gradient (HVPG = WHVP - FHVP) which is similar to portal pressure in alcoholic cirrhosis (27) but lower than portal pressure in nonalcoholic cirrhosis (28).

A quantitative estimate of esophageal collateral circulation can be obtained by use of continuous thermodilution measurements in the azygos vein (29), the site of drainage of esophageal varices, but is said to be no better than portal pressure itself at predicting variceal bleeding.

Five studies have examined the systemic and portal hemodynamic effects of nitroglycerin in patients with cirrhosis (Table 1) and three showed a reduction of HVPG following nitroglycerin administration (31, 32, 35). The effect on azygos blood flow was highly variable including some individuals in whom flow increased,

suggesting vasodilation of portasystemic collaterals. Mechanisms which might be responsible for this variable response include; 1. Vasorelaxation at sinusoidal and collateral level leading to reduced resistance to flow through the portal-collateral circulation and reduced portal pressure. 2. Relaxation of arterial smooth muscle lowering the arterial blood pressure and triggering high-pressure arterial baroreceptors to cause reflex splanchnic vasoconstriction. This would reduce portal inflow and hence portal pressure. 3. Systemic venodilation reducing the cardiac preload and thus activating low-pressure cardiopulmonary baroreceptors. This would also cause reflex splanchnic vasoconstriction, reducing the portal venous inflow and thus reducing portal pressure.

An important aspect of the acute nitroglycerin studies in man (Table 1) is the difference in doses used and the hemodynamic effect of different doses of nitroglycerin has been found to be heterogeneous (Table 1). This suggests that different doses might act by different mechanisms and raises questions about the comparability of these studies.

Seven groups have examined the acute hemodynamic effects on cirrhotic patients given isosorbide dinitrate by a variety of routes (Table 2). Only one group (37) showed no effect on portal pressure; all others showed a reduction in HVPG which was accompanied by a fall in cardiac index and mean arterial blood pressure. The reduction of HVPG was achieved by a significant fall in WHVP rather than a rise in FHVP.

Table 3 shows that Is-5-Mn has the same hemody-

TABLE 1
Acute Effect of Nitroglycerin Administration

	Staritz 1985 (30)	Garcia-Tsao 1987 (31)	Westaby 1988 (32)	Moreau 1989 (33)	Rector 1990 (34)	Iwao 1991 (35)
No. of patients	14	11	12	11	10	14
Dose, route, time	(1.2-mg spray onto tongue)	(0.6 mg SL 2-12 min peak change)	(200 µg/min iv peak change)	(7-15 µg/min iv peak change)	(40 µg/min iv peak change)	(Transdermal tape 10 mg for 60 min)
SVRI*			1887-1784	1537-1581		
WHVP		22.5-18.9†	28.7-25.8†	29.2-29.3	28-27	26.0-22.1†
HVPG		17.9-15.1†	16.4-13.3†	20.9-21.1	16-16	17.9-15.1†
AzBF		604-437		0.53-0.55	0.46-0.46	
ELBF				1.34-1.10		925-919
HR		73-83†	84-97	82-89.5†	84-87	72-71
MABP		96-76†	95-79†	86.8-79.4†	89-74†	106.1-91.3†
CO/CI			4.11-3.29†	4.29-3.9†	7.0-6.3†	
(a) (b)			(b)	(b)	(a)	
IOVP Grade III varices	22.8-12.0†					
IOVP Grade II varices	16.3-10.0†					

* SVRI, systemic vascular resistance index in dynes/s per m²; WHVP, wedged hepatic venous pressure in mm Hg; HVPG, wedged minus free hepatic venous pressure in mm Hg; AzBF, azygos blood flow in ml/min; ELBF, estimated liver blood flow in ml/min (ICG method); HR, heart rate in beats/min; MABP, mean arterial blood pressure in mm Hg; CO/CI, cardiac output/cardiac index in L/min; IOVP, interestesophageal variceal pressure in mm Hg.

† Denotes significant difference, at least at 95% level.

TABLE 2
Acute Effect of Isosorbide Dinitrate Administration

	Hallems 1983 (36)	Dawson 1985 (37)	Freeman 1985 (38)	Merkel 1987 (39)
No. of patients	10	8	10	13
Dose, route, time	5 mg SL 10 min 1964-1803	5 mg oral 30 min	10 mg iv 30 min	5 mg SL* 20-30 min 2080-1848†
SVRI†				24.8-15.6†
WHVP			19.4-12.5†	20.3-13.5†
HVPG	17.3-13.6†	22-23	14.7-8.6†	
AzBF				
ELBF				1384-1284
HR	86-88			83-87†
MABP	81-65†	123-88 (systolic)		100-73†
CO/CI	3.7-3.2†			3.92-3.26†
(a) (b)	(b)			(b)

Acute Effect of Isosorbide Dinitrate Administration					
	Blei 1987 (12)	Qureshi 1988 (40)	Mols 1989 (41)	Bhatia 1990 (42)	Merkel 1990 (43)
No. of patients	7	8	6	17	13
Dose, route, time	10 mg oral 1 h 4 h	40 mg oral 1 h 4 h	5 mg SL 20 min	5 mg SL 15 min	5 mg SL 20 min
SVRI				1654-1537	
WHVP	22.2-20.5† 22.2-21.4	23.7-20.1† 23.7-20.7†	24.6-18.5†	28-24†	
HVPG	15.4-12.3† 15.4-13.1	16.6-13.7† 16.6-13.9†		18-14†	
AzBF					
ELBF				650-710	
HR	70-72	82-86	86-94†	92.5-102.6†	No change in plasma clearance of ICG
MABP	90-76†	89-72†	89-74†	85.8-80.4†	
CO/CI	4.71-3.63†	4.92-4.02†	4.2-3.7		
(a) (b)	(b)	(b)	(b)		
Intrasplenic pulp pressure				43.6 → 35.6†	

* SL, sublingual.
† SVRI, systemic vascular resistance index in dynes/s per m²; WHVP, wedged hepatic venous pressure in mm Hg; HVPG, wedged minus free hepatic venous pressure in mm Hg; AzBF, azygos blood flow in ml/min; ELBF, estimated liver blood flow in ml/min (ICG method); HR, heart rate in beats/min; MABP, mean arterial blood pressure in mm Hg; CO/CI, cardiac output/cardiac index in L/min.
‡ Denotes significant difference, at least at 95% level.

TABLE 3
Acute Effect of Is-5-Mn Administration

	Hayes 1988 (44)	Tsai 1989 (45)	Navasa 1989 (46)
No. of patients	11	10	23
Dose, route, time	20 mg Is-5-Mn 60 min	20 mg Is-5-Mn 60 min	20 mg or 40 mg Is-5-Mn 60 min
SVRI*	1831-2030†	1131-1040	899-994†
WHVP	33.3-29.8†	21.8-21.0	785-746
HVPG	23.9-21.8†	17.8-16.3	23.5-21.9†
AzBF			18.3-16.5†
ELBF	1940-1639†		0.52-0.51
HR	85-89.7†	71-72	890-1003†
MABP	90-88.9	92-82†	0.74-0.62†
CO/CI	3.89-3.42†	4.1-3.9	1320-1230
(a) (b)	(b)		80.4-90.7
			88.4-81.3†
			8.0-6.8†
			8.9-7.7†
			(a)

* SVRI, systemic vascular resistance index in dynes/s per m²; WHVP, wedged hepatic venous pressure in mm Hg; HVPG, wedged minus free hepatic venous pressure in mm Hg; AzBF, azygos blood flow in ml/min; ELBF, estimated liver blood flow in ml/min (ICG method); HR, heart rate in beats/min; MABP, mean arterial blood pressure in mm Hg; CO/CI, cardiac output/cardiac index in L/min.
† Denotes significant difference, at least at 95% level.

namic behavior as the dinitrate, with the exception of one study which included mainly Child's A patients (45).

An illustration of different dose-dependent mechanisms of portal hypotensive action may be seen by the work of Nevasa *et al.* (46). The changes in cardiac output and systemic resistance were similar with either 20 or 40 mg Is-5-Mn but after 40 mg Is-5-Mn there were significantly greater reductions in portal pressure, arterial blood pressure, and azygos blood flow.

These results are divergent from the study of Hayes *et al.* (44) which indicated that following administration of 20 mg Is-5-Mn the fall in portal pressure was associated with a fall in liver blood flow secondary to baroreceptor mediated splanchnic vasoconstriction, a response only observed with 40 mg of nitrate in Nevasa's study. Differences between studies may be related to different severities of liver disease (and different volumes of distribution of nitrate), different degrees of portasystemic shunting, timing of Is-5-Mn administration or different variceal bleeding histories. Other confounding variables include impaired cardiovascular responsiveness (33), baseline cardiac filling pressure (34), and severity of liver disease (47).

Despite the potential complexity of action of nitrates in portal hypertension, the overall results of studies of acute administration of nitrates have been remarkably uniform (Tables 2 and 3). The fall in portal pressure seen after nitrates compares favorably with the effect of propranolol but that, following nitrate administration, the fall in HVPG was achieved by a fall in WHVP alone [unlike propranolol which reduces HVPG by both a fall in WHVP and a rise in FHVP (48-50)]. Reducing WHVP may be more important than reducing HVPG in protection against variceal bleeds (51, 52).

EFFECT OF ORGANIC NITRATES ON LIVER BLOOD FLOW AND HEPATIC RESISTANCE

Liver blood flow in hemodynamic studies is usually estimated by indocyanine green dye (ICG) extraction (53, 54). Calculations of hepatic resistance from data in Tables 1-5 suggest that administration of organic nitrates, either acutely or chronically, induces a significant fall in portal pressure that is due largely to a reduction in resistance rather than to a reduction in liver blood flow (which would be undesirable) although such calculation is of limited value as the variable component of hepatic arterial flow is unknown. The implication of the hepatic resistance calculation, however, is either that portal flow is not reduced or that a compensatory increase in hepatic arterial flow takes place, *i.e.*, reciprocity.

EFFECT OF ORGANIC NITRATES ON AZYGOS (COLLATERAL) BLOOD FLOW

The rationale for the pharmacologic treatment of esophageal varices was originally based on the observation that drugs could reduce portal pressure (59). Propranolol, the first drug studied, reduces the portal pressure gradient in only 60% of patients with cirrhosis but reduces variceal flow, as measured by azygos flow, in all (59). It remains unclear whether the major benefit of propranolol, which reduces primary and secondary variceal bleeding (60), is by portal pressure reduction or reduced azygos flow. Since the portal pressure reduction is small and variable, the effect on collateral (azygos) flow may well be more important.

Nevasa *et al.* (46) showed that azygos blood flow remained unchanged after administration of 20 mg isosorbide-5-mononitrate despite a significant reduction in portal pressure and cardiac output. As the efficacy of vasoactive drugs in either controlling an acute variceal bleed or preventing recurrent hemorrhage may be related to their ability to reduce blood flow through collateral vessels, determination of the effects of vasoactive agents on both collateral blood flow and portal pressure is important in evaluating their therapeutic potential. In this respect the change in azygos flow to nitrates is seen to be highly variable and probably dose-dependent (Tables 2-5) and it would seem important to find criteria, preferably noninvasive, *e.g.*, blood pressure or heart rate changes, which would make possible the selection of a dose of nitrate for an individual that both reduces portal pressure and azygos blood flow in order to properly assess its efficacy in reduction of variceal bleeding in future clinical trials.

Chronic effect of organic nitrates—Is tolerance a problem?

Tachyphylaxis to nitrate preparations is well recognized in patients with heart disease given nitrates for 18 h or longer (61, 62). Organic nitrates are metabolized by vascular smooth muscle as well as by the liver (63) and tolerance is associated with an alteration of vascular metabolism of the nitrate administered (63, 64); systemic clearance of administered nitrate diminishes and the systemic arteriovenous nitrate gradient narrows, *i.e.*, with long-term dosing plasma nitrate concentrations rise over time. The cyclic-GMP response to nitrates is also diminished in the presence of tolerance.

Administration of exogenous thiols, *e.g.*, *N*-acetylcysteine, enhances the nitrate vascular effects in the nontolerant state (65) and removal of organic nitrates from contact with the blood vessel wall for a period of time restores vascular responsiveness. Studies with isolated rat abdominal aortic rings (with endothelium) showed they retained the repair mechanism to overcome tolerance without the need for exogenous factors,

which suggests regulation of vascular responsiveness is at the vascular level. Nitrate tolerance appears to be related to reduced sulphhydryl availability within vascular tissue and sulphhydryl groups are required for vasodilation.

Experience to date (Table 4 and 5) shows that a sustained fall in portal pressure is seen after chronic nitrate administration in cirrhotic patients, which suggests that full tolerance is not of concern in such patients, although partial tolerance has been reported (58).

A recent report suggests that higher than normal concentrations of *N*-acetylcysteine are found in the urine of patients with chronic liver disease and it would be interesting to speculate that endogenous *N*-acetylcysteine might be responsible for patients with cirrhosis having less tolerance to nitrates than normal controls/cardiatic patients (66).

Clinical studies with cardiac patients indicate that tolerance can be reversed by a nitrate free/nitrate poor dosing interval (67, 68). It would seem a reasonable precaution to observe these rules in prescription of nitrates for patients with cirrhosis.

THERAPEUTIC POTENTIAL OF ORGANIC NITRATES IN LIVER DISEASE

Nitrates in the management of acute variceal bleeding

The most effective treatment for an acute variceal bleed is injection sclerotherapy (69) or banding (70, 71). Either is effective only if promptly applied by experienced personnel. Balloon tamponade has been

shown to be as effective as sclerotherapy (72), but is similarly only effective in proficient hands; many patients tolerate the balloon poorly and rebleeding within hours of balloon removal is common (73).

It would therefore be desirable if drug therapy was easy to use and efficacious in controlling bleeding, particularly in nonspecialist centers. The first agent used, vasopressin, is not particularly effective and has serious side effects (74-76).

Several studies have shown that the addition of nitrates to vasopressin produces additional reduction in portal pressure at the same time counteracting some of the deleterious systemic hemodynamic effects such as reduced cardiac output and hypertension (32, 77-80).

Glypressin, an analogue of vasopressin (81), may have less side effects than vasopressin (82) but no study comparing them with vasopressin plus nitrates has yet been reported.

Transdermal nitrate patches have been advocated but much more reliable plasma nitrate levels are obtained by the intravenous route (63), especially in a shocked patient with compromised skin blood flow (83). In the acute setting, vasopressin should be used at a dose of 0.4-0.8 U/min over 12 h plus a nitroglycerin infusion at 0.2 mg/min for the duration of the vasopressin infusion.

Nitrates for the prevention of rebleeding or prophylaxis of first hemorrhage

Propranolol reduces the frequency of variceal hemorrhage (84) but has less influence on mortality rates

TABLE 4
Chronic Effect of Isosorbide Dinitrate Administration

	Freeman 1985 (38)	Cervinka 1989 (55)	Ikegami 1992 (56)	Vorobioff 1992 (57)
No. of patients	6	10	9	10
Dose, route, time	20 mg qds 30 days 3-4 h after AM dose of drug	80 mg/day slow release for 14 days	40 mg/day for 4 wk	15 mg/day, increasing every 3-4 days to maximum of 82 ± 10 mg/day dura- tion of rx 65 ± 23 days
SVRI*			1308-1178	
WHVP	18.9-9.8†	32.9-17.9†	23.7-21.3†	25.7-22.5†
HVPG	12.6-5.4†	20-9.0	15.6-12.8†	18.9-16.5
AzBF				
ELBF			720-710	
HR		"increased 3-5 bpm"	73-71	87-82
MABP		80.3-76.8	97-87.0†	95-90
CO/CI			5.7-5.7	
(a) (b)			(a)	

* SVRI, systemic vascular resistance index in dynes/s per m²; WHVP, wedged hepatic venous pressure in mm Hg; HVPG, wedged minus free hepatic venous pressure in mm Hg; AzBF, azygos blood flow in ml/min; ELBF, estimated liver blood flow in ml/min (ICG method); HR, heart rate in beats/min; MABP, mean arterial blood pressure in mm Hg; CO/CI, cardiac output/cardiac index in L/min.

† Denotes significant difference, at least at 95% level.

TABLE 5
Chronic Effect of Is-5-Mn Administration

	Tsai 1989 (45)	Garcia-Pagan 1990 (58)
No. of patients	6	11
Dose, route, time	20 mg Is-5-Mn b.d., 1 week	40 mg Is-5-Mn b.d., 3 months
SVRI*	1131-1177	950-915
WHVP	21.8-18.5	25.5-24.9
HVPG	17.8-15.2	18.6-17.2†
AzBF		0.58-0.52
ELBF		1320-1510†
HR	71-68	77-73†
MABP	92-91	89.4-82.6†
CO/CI	4.1-3.6	7.5-7.2
(a) (b)	(b)	(a)

*SVRI, systemic vascular resistance index in dynes/s per m²; WHVP, wedged hepatic venous pressure in mm Hg; HVPG, wedged minus free hepatic venous pressure in mm Hg; AzBF, azygos blood flow in ml/min; ELBF, estimated liver blood flow in ml/min (ICG method); HR, heart rate in beats/min; MABP, mean arterial blood pressure in mm Hg; CO/CI, cardiac output/cardiac index in L/min.

† Denotes significant difference, at least at 95% level.

than might be expected (60). Experience of use of nitrates in these two clinical situations is still limited; as yet, no data on mortality are available.

We would suggest that nitrates should be considered for treatment of portal hypertension in patients for whom β -blockade is contraindicated. Because they reduce portal pressure in virtually all patients with cirrhosis, unlike propranolol, nitrates might even hold significant advantages over β -blockade. However, their use cannot be confidently recommended until randomized, controlled clinical trials are performed.

Combination therapy for prophylaxis

Garcia-Pagan *et al.* (85) studied the effect of propranolol *versus* propranolol plus nitrate. At 3 months, the HVPG decreased more in patients given both nitrate and β -blocker than those given β -blocker alone. The two treatment regimens caused similar reductions in azygos flow and cardiac output.

There were significant falls in liver blood flow and ICG clearance after propranolol therapy, but not after combined therapy, and acute administration of Is-5-Mn enhanced the portal pressure reduction by propranolol (85). Whether the greater hemodynamic effect translates into better clinical efficacy remains to be determined by randomized controlled trials.

Compliance

Rates of compliance have been variously estimated at 10-90%, and depend on many factors including the enthusiasm of the doctor, the disease being treated, and the patients perception of importance of the disease (86).

Caron and Roth (87) showed that doctors could not

predict their patient compliance more accurately than by chance alone, so if compliance is to be accurately assessed, specific methods (*e.g.*, tablet counts, blood levels) must be used. It is therefore surprising that only one chronic nitrate study to date (58) has made any attempt to assess compliance, despite the aim to assess any potential tolerance to nitrates.

Compliance can be improved by simplifying therapeutic regimes, using drugs with few side effects—perhaps in calendar packs, and thorough explanation and education for the patient. This is a major challenge in the field of chronic liver disease.

SUMMARY

In conclusion, chronic administration of organic nitrates, allowing for a nitrate-free interval each day, to cirrhotic patients reduces portal pressure with a variable, dose-dependent effect on azygos blood flow. The mechanisms of portal pressure reduction may vary according to the different doses and severity of liver disease. No studies to date have used dose-titration to determine the optimal beneficial effects in an individual patient, but some have at least compared the effect of different doses. Perhaps an end-point of mean arterial blood pressure reduction to 70 mm Hg could be suggested for future studies.

The prophylactic benefit of nitrates for variceal bleeding still remains to be tested by controlled clinical trials. However, several problems in this evaluation need to be addressed, including patient compliance, selection of responders, evaluation of treatment, dose, and the duration of treatment.

In contrast to the diminished expectations of propranolol for the prevention of rebleeding, there is mounting evidence of a major role for nitrates.

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Portal and systemic haemodynamic action of *N*-acetylcysteine in patients with stable cirrhosis

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Abstract

The effects of intravenous *N*-acetylcysteine on hepatic and systemic haemodynamics were investigated in 11 patients with stable cirrhosis (eight alcohol; two primary biliary cirrhosis; one cryptogenic). *N*-acetylcysteine administration had no effect on the mean heart rate or mean arterial blood pressure despite a significant fall in systemic and pulmonary vascular resistance. Cardiac index increased but estimated liver blood flow and portal venous pressure did not change significantly. Administration of *N*-acetylcysteine resulted in increased oxygen delivery to the tissues because of the increased cardiac index but this was not accompanied by a rise in either arteriovenous oxygen extraction ratio or mean tissue oxygen consumption. Therefore *N*-acetylcysteine administration seems to confer no haemodynamic benefit to patients with cirrhosis.

(Gut 1994; 35: 1290-1293)

N-acetylcysteine (NAC), a sulphurated amino acid, has been reported to increase cardiac output, tissue oxygen delivery, and utilisation in fulminant hepatic failure.¹ As some of the haemodynamic changes seen in patients with cirrhosis are similar to those with fulminant hepatic failure, *N*-acetylcysteine may also increase tissue oxygen use and delivery in patients with cirrhosis. The aim of this study was to determine the haemodynamic effects of this agent in cirrhosis with particular reference to oxygen delivery and consumption.

Methods

Eleven cirrhotic patients proved by biopsy (eight men; three women; mean age 61.5 years; range 43-74) with a range of disease severity (4A, 3B, 4C Child's-Pugh grades) and aetiology (two primary biliary, one cryptogenic, and eight alcoholic cirrhosis) were studied. Exclusion criteria included known myocardial infarction or ischaemic heart disease, valvular heart disease, pregnancy, vasoactive treatment, current viral hepatitis B, C, or D or bleeding diathesis with prothrombin time ratio (PTR) greater than 2.5:1. The eight alcoholic cirrhotic patients had no evidence of alcoholic cardiomyopathy and were not actively drinking or withdrawing from alcohol as monitored by liver function tests, breath alcohol concentration, and clinical state. All patients gave witnessed

informed consent and ethical permission was obtained from Lothian Health Board medical ethics subcommittee.

Each patient fasted after a light breakfast on the day of the study. In the catheter laboratory a 7.5 F introducer (Edwards, Critical Care Division, Irvine, USA) was placed in the right femoral vein under local anaesthesia (2% lignocaine). A Swan-Ganz catheter (7 F Edwards, Irvine, USA) was inserted under fluoroscopic screening and continuous pressure recording into the pulmonary artery. The tip of the catheter was positioned in a major branch of the pulmonary artery. The mean heart rate (from ECG monitor, Hewlett Packard HP monitoring system, Germany) and mean arterial blood pressure (manual sphygmomanometer) were checked every five minutes initially until all patients had achieved haemodynamic stability for at least 20 minutes. Simultaneous femoral artery, pulmonary artery, and femoral vein blood gas samples were then taken into heparinised blood gas syringes and analysed immediately for oxygen saturation (Co-Oximeter 282, Instrumentation Laboratory, Lexington, MA, haemoglobin, and oxygen tension (PO₂) (ABL 2000, Radiometer, Copenhagen). The pulmonary artery free and wedge pressure and cardiac output (by Swan-Ganz thermodilution method using 10 ml of cold 5% dextrose as injectate) were estimated. Cardiac output was measured in quadruplicate and the results expressed as a mean value. Cardiovascular pressures were measured with reference to the mid axillary line. *N*-acetylcysteine (Parvolex, Duncan Flockhart, Greenford, UK) was infused intravenously by accurate infusion pump (Gemini 2, IVAC, USA) at 150 mg/kg in 200 ml 5% dextrose over 15 minutes followed by 15 minutes infusion at 125 ml/h of 50 mg/kg in 500 ml 5% dextrose. The blood gas analysis, pulmonary artery pressures, mean arterial blood pressure, cardiac output, and heart rate were remeasured 30 minutes after the start of *N*-acetylcysteine (NAC) infusion - that is, during infusion. Derived haemodynamic variables for each patient were calculated according to standard formulas.²

The O₂ content was calculated according to the formula;

$$(\text{Hb} \times 1.34 \times \text{sat \%} + \text{O}_2 \text{ tension} \times 0.0031).$$

The delivery of oxygen to tissues was calculated as the product of the cardiac index and the arterial oxygen content. Oxygen consumption was calculated from the reverse Fick

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TABLE I Effect of N-acetylcysteine (NAC) infusion on mean haemodynamic parameters in 11 patients with stable cirrhosis (eight alcohol; two PBC; one cryptogenic)

Variable	Before NAC	After NAC	p Value
HR	78.3 (3.2)	79.2 (2.7)	NS
MABP	88 (2.7)	85.8 (3.3)	NS
SVRI	1902 (195)	1645 (193)	p<0.02
PVRI	169.2 (28)	127 (21)	p<0.01
O ₂ delivery	1086 (136)	1214 (140)	p<0.01
LVSWI	60.3 (7.3)	65.9 (10.3)	p<0.05
CI	3.85 (0.35)	4.36 (0.36)	p<0.01
O ₂ consumption	240 (18)	240 (14.5)	NS
OER	23.6 (2.1)	21.3 (1.7)	NS (p>0.05)

NAC infusion was at 150 mg/kg body weight in 200 ml 5% dextrose for 15 minutes followed by 15 minutes of 50 mg/kg in 500 ml 5% dextrose (set at 125 ml/h). Mean values (SEM) are shown. HR is heart rate in beats per minute, MABP is mean arterial blood pressure in mm Hg, SVRI and PVRI are systemic and pulmonary vascular resistance index respectively in dynes \times sec/cm⁵ \times m². O₂ delivery and consumption are measured in ml/min. CI is cardiac index in l/min/m² and LVSWI is left ventricular stroke work index in g \times m/m². OER is the oxygen extraction ratio expressed as a percentage. Statistical significance is taken at the 95% value.

TABLE II Effect of N-acetylcysteine infusion at 150 mg/kg body weight for 15 minutes in 200 ml 5% dextrose followed by 15 minutes of 50 mg/kg in 500 ml 5% dextrose (set at 125 ml/h) on mean blood gas values in 11 patients with cirrhosis (eight alcohol; two PBC; one cryptogenic)

	Before NAC	After NAC	p Value
Femoral artery PO ₂ (kPa)	11.25	10.32	NS
Femoral artery O ₂ saturation (%)	94.3	93.7	NS
Pulmonary artery PO ₂ (kPa)	5.35	5.37	NS
Pulmonary artery O ₂ saturation (%)	72.1	73.8	NS
Femoral vein PO ₂ (kPa)	5.15	5.70	NS
Femoral vein O ₂ saturation (%)	67.9	74.9	NS

Statistical significance is taken at the 95% value.

TABLE III Effect of N-acetylcysteine infusion on estimated liver blood flow, wedged, and free hepatic pressures and hepatic venous pressure gradient in six of the 11 (randomly selected) patients with cirrhosis

Variable	Units	Before NAC	After NAC	p Value
ELBF	ml/min	1161 (167)	1283 (92)	NS
WHVP	mm Hg	20.7	22.2	NS
FHVP	mm Hg	7.3	8.5	NS
HVPG	mm Hg	13.3	13.7	NS

N-acetylcysteine infusion was initially at 150 mg/kg body weight in 200 ml 5% dextrose for 15 minutes followed by 50 mg/kg in 500 ml 5% dextrose (at 125 ml/h). Figures are expressed as means. A significance value of more than 95% is taken. ELBF is estimated liver blood flow, WHVP is wedged hepatic venous pressure, FHVP is free hepatic venous pressure, and HVPG is hepatic venous pressure gradient.

TABLE IV Control data. The haemodynamic response of five of 11 patients with cirrhosis to 5% dextrose infusion only

	Baseline	After 5% dextrose alone	p Value
Heart rate (bpm)	74.4 (4.6)	73.2 (41)	NS
MABP (mm Hg)	91 (3.3)	89.2 (3.1)	NS
RAP (mm Hg)	3.8 (0.37)	3.8 (0.37)	NS
CI (l/min/m ²)	3.46 (0.30)	3.54 (0.26)	NS
SVRI (dynes \times sec/cm ⁵)	2064 (184)	2008 (183)	NS
LVSWI (g \times m/m ²)	59.0 (7.8)	60.2 (5.3)	NS
PVRI (dynes \times sec/cm ⁵)	188 (44)	184.7 (48.5)	NS

Values are expressed as mean (SEM). MABP is mean arterial blood pressure, RAP is right atrial pressure, CI is cardiac index, SVRI and PVRI are systemic and pulmonary vascular resistance index respectively. LVSWI is left ventricular stroke work index. Statistical significance is taken at the 95% value.

equation (cardiac index \times a-v O₂ difference); this technique has been validated against the direct method of calculation of O₂ consumption by analysis of respired gas.³ The oxygen extraction ratio was calculated by dividing the difference between the arterial and venous O₂ content by the arterial O₂ content.

The hepatic venous pressure gradient (wedged hepatic venous pressure - free hepatic venous pressure) and estimated liver blood

flow (ICG, Cardiogreen Hynson, Westcott and Dunning Inc, Baltimore, MD - continuous infusion method^{4,5}) were measured immediately before and after NAC infusion randomly in six of 11 patients with cirrhosis. For hepatic pressure measurements a sidewinder II catheter (Cordis, USA) was inserted into the main right hepatic vein under fluoroscopic control. Inflating and releasing the balloon permitted repeated consistent measurements of wedge and free hepatic pressure respectively to be made. The intravenous infusion of indocyanine green was made up in saline and infused at a rate of 0.20 mg/m²/min after a priming dose of 0.20 mg/kg body weight. After a 30 minute equilibration period three simultaneous samples of peripheral and hepatic venous blood were drawn into heparinised syringes for the determination of estimated liver blood flow. The indocyanine green concentrations in the serum and infusion fluid were estimated by spectrophotometry at 810 nm in a Zeiss PMQ II spectrophotometer against a blank serum sample. The estimated liver blood flow was derived from the equation estimated liver blood flow = R/(A-H) (1-PCV), where R is the rate of infusion of indocyanine green in mg/min; A and H are the concentrations of dye in peripheral and hepatic venous blood in mg/l; and PCV is the packed cell volume.⁴⁻⁷

As the systemic haemodynamic effect of NAC has been assessed previously in patients without liver disease it was felt that inclusion of normal healthy controls in an invasive study such as this was unethical.^{8,9} The haemodynamic response, however, to infusion of the same volume of 5% dextrose as above but not containing N-acetylcysteine to five (randomly selected) patients with cirrhosis was assessed to investigate any potential volume loading effect of the infusion.

STATISTICAL ANALYSIS OF HAEMODYNAMIC VARIABLES

The results after the infusion of NAC were compared with the baseline haemodynamic values before infusion. All results were expressed as mean (SEM) with statistical analysis by a two tailed paired Student's *t* test.

Results

Administration of N-acetylcysteine had no effect on mean heart rate or arterial blood pressure (Table I). Infusion of NAC resulted in vasodilatation with a significant reduction in both mean systemic vascular resistance index and pulmonary vascular resistance index (Table I). No significant changes in right atrial pressure, pulmonary artery free and wedge pressure occurred.

Administration of NAC resulted in a significant increase in mean oxygen delivery resulting from an increase in the mean cardiac index (Table I). This was associated with a significant increase in left ventricular stroke work index (Table I). Both arteriovenous oxygen extraction ratio and the mean oxygen consumption

did not rise in response to NAC (Table I).

The effect of *N*-acetylcysteine on mean blood gas values showed a minor fall in arterial oxygen tension and increase in venous oxygen tension after NAC administration (Table II).

There was no significant statistical correlation between Child's score variables – that is, serum albumin, bilirubin, and prothrombin ratio – and degree of change in response to NAC of cardiac index, oxygen delivery, oxygen consumption, oxygen extraction ratio or blood gases.

Mean estimated liver blood flow increased in response to NAC infusion; but did not achieve statistical significance (Table III). Wedged hepatic venous pressure, free hepatic venous pressure, and portal pressure showed no significant change after *N*-acetylcysteine infusion (Table III).

The control group of five patients who received intravenous dextrose alone showed no significant change in haemodynamic parameters (Table IV).

Discussion

We have shown that NAC acts as a vasodilator in patients with cirrhosis, similar to the effect shown in fulminant hepatic failure.¹ This has not occurred in patients with cardiac failure, with symptoms of chest pain or in those fully recovered from acute liver failure.^{1,8,9} NAC is not known to possess direct relaxant activity in normal vascular smooth muscle¹⁰ and given to pigs, both healthy and with septic shock produced no significant haemodynamic changes.^{11,12} There is thus convincing evidence that NAC has no haemodynamic action in healthy controls. As NAC given to our 11 cirrhotic patients clearly conferred no haemodynamic benefit we felt that to study more patients would be unethical.

The five patients in our control group showed no vasodilation in response to dextrose infusion alone (Table IV). Thus the postulate that the haemodynamic changes in the 11 patients given *N*-acetylcysteine infusion were caused by the effects of volume loading or patients relaxing after the initial invasive procedures can be ruled out.

In patients with cirrhosis, unlike those with fulminant hepatic failure,¹ the mean arterial pressure did not change and the oxygen extraction ratio and O₂ consumption did not rise in response to NAC infusion (Table I). The cardiac index, systemic vascular resistance index, pulmonary vascular resistance index, stroke work index, and O₂ delivery % changes were, however, similar between cirrhotic and fulminant hepatic failure patients (Table I).

As most drugs that primarily cause afterload reduction increase cardiac index without affecting left ventricular stroke work index (or rate pressure product, or other measures of myocardial consumption) the rise in cardiac index in association with left ventricular stroke work index seen here is suggestive of a positive inotropic response rather than purely peripheral vasodilation and reduction in left ventricular afterload.

Interestingly, there were no reported

changes in arterial PO₂, calculated a-v tension gradient, and shunt in hepatic failure patients in response to NAC.¹ Errors in estimation of mixed venous O₂ saturation and tension could occur if the blood is drawn rapidly or if the catheter is positioned peripherally within the pulmonary artery because of contamination of the mixed venous pulmonary blood with arterIALIZED blood drawn from the pulmonary capillaries and veins. We are confident that sufficient care was taken to prevent this error occurring in our study. This is supported by the increasing PO₂ trend seen in peripheral vein samples after NAC infusion, paralleling the increase in pulmonary artery PO₂ after the infusion (Table II).

We believe that the difference between the action of NAC to increase O₂ delivery in cirrhosis but not tissue O₂ consumption (compared with fulminant hepatic failure) may be because patients with cirrhosis have established pulmonary shunts¹³ and NAC acts as a vasodilator to increase pulmonary shunting. This hypothesis is supported by the finding that pulmonary vascular resistance (Table I) fell in response to NAC infusion. The exact nature of such shunting is controversial.^{14–18} The multiple inert gas elimination technique¹⁹ has also shown V/Q mismatching in some patients with cirrhosis. Patients with lower pulmonary vascular resistance have greater V/Q mismatch.¹³ Our data suggest that cirrhotic patients, unlike hepatic failure¹ and cardiac failure²⁰ patients, are not dependent on supply of oxygen to increase consumption. This may, however, just reflect the well compensated liver disease in most of these patients and it might be necessary to evaluate a group of grade C patients alone before a beneficial effect of NAC can be excluded.

Differences between fulminant hepatic failure patients and our cirrhotic patients may also result from the fact that some of the fulminant patients had encephalopathy (with and without neurohumoral factors) and were on mechanical ventilation (volume controlled) with continuous intravenous infusion of muscle relaxant (atracurium 50 mg/h).¹ Pulmonary capillary wedge pressure had to be stabilised at 8–14 mm Hg by infusion of 4.5% human albumin solution before the study.

Sulphydryl groups are required for the relaxation of vascular smooth muscle. Packer's data⁸ support the hypothesis that sulphydryl depletion plays a part in the development of tolerance to nitroglycerin and as NAC, and other sulphydryl donors, can reverse this tolerance we postulate that in cirrhotic patients who are known to be deplete in sulphurated amino acids²¹ NAC acts by sulphydryl repletion to cause vasodilatation. NAC may be acting directly on vasculature or indirectly by nitric oxide by sulphydryl donation.²² As partial tolerance to organic nitrates (used to reduce portal hypertension in cirrhotic patients) has been reported²³ we would speculate that should longterm tolerance be a problem NAC or its oral derivative methionine might reverse this tolerance and thus be of therapeutic value.

It should be appreciated that not all vasodilators have the same haemodynamic effect in patients with cirrhosis, for example, nitrates cause vasodilatation but cause a fall in liver blood flow, cardiac output, and mean arterial blood pressure and an increase in systemic vascular resistance²⁴; this presumably reflects different mechanisms or sites of action of vasodilating agents.

N-acetylcysteine given to patients with cirrhosis is probably not of haemodynamic value. This study illustrates that extrapolation of drug use between patients with fulminant hepatic failure and chronic liver disease is not always justifiable. We would still advocate its use, however, in paracetamol poisoning, particularly in patients with chronic alcohol intake (as such patients have increased susceptibility to paracetamol).^{25,26}

This work has been presented in part at the British Society of Gastroenterology meeting 24–26th March 1993 and appears in abstract form in *Gut* 1993; 34 (suppl): S43.

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Portal and systemic haemodynamic response to acute and chronic administration of low and high dose isosorbide-5-mononitrate in patients with cirrhosis

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Abstract

Oral isosorbide-5-mononitrate (Is-5-Mn) was given in doses of 10 and 40 mg acutely and chronically (twice daily for four weeks), allowing a nitrate free interval to 25 patients with cirrhosis. Both 10 mg and 40 mg Is-5-Mn reduced the hepatic venous pressure gradient acutely and chronically, without evidence of tolerance. This was achieved by a reduction in the wedged hepatic venous pressure. The effect on mean azygos blood flow was variable with no significant mean change seen acutely or after chronic use with either dose. The variability was dependent not on the dose used but on the initial azygos flow; the flow in patients with initially low values increased and those with high azygos flows decreased after nitrate challenge. The development of the porto-collateral flow seems an important parameter in predicting haemodynamic response to Is-5-Mn.

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Keywords: cirrhosis, isosorbide-5-mononitrate, hepatic venous pressure.

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Variceal haemorrhage is a major cause of morbidity and mortality in cirrhosis and drug treatment of portal hypertension aims to reduce this. Previous reports show that both isosorbide dinitrate¹⁻¹² and isosorbide-5-mononitrate (Is-5-Mn)¹³⁻¹⁷ are capable of reducing the hepatic venous pressure gradient or azygos vein (collateral) blood flow, or both, both acutely and with chronic use but the results have been highly variable. For example we, and others, have found that azygos blood flow may increase in some patients in response to nitrate.¹⁵⁻¹⁷

This study aimed to examine this variability to establish whether it was related to nitrate dose or patient factors. In addition, we wished to establish whether there was any evidence of a tolerance effect with Is-5-Mn in patients with cirrhosis.

Methods

STUDY POPULATION

Twenty five patients with cirrhosis proved by liver biopsy (14 men; 11 women) and with a range of disease severity (eight A; 11 B; six C Child's-Pugh grading) were studied. Exclusion criteria included known myocardial infarction or ischaemic heart disease; valvular heart disease; cardiomyopathy; cerebrovascular disease; pregnancy; vasoactive medication; current viral hepatitis B, C, or D; or bleeding diathesis with prothrombin time ratio greater than 2.5:1. All patients gave written informed consent and ethical permission was obtained from the Lothian Health Board Medical Ethics Subcommittee. All studies conformed to the Helsinki declaration. Each patient was randomised to receive either 10 mg or 40 mg Is-5-Mn by random number selection within sealed envelopes. From Table I it may be seen that the two dosage groups were not significantly different except with regard to sex ratios.

STUDY PROCEDURE

Each patient fasted after a light breakfast on the day of the study. Three lead (lead II) ECG monitoring was begun by attachment to a Hewlett Packard HP monitoring system (Germany), and mean arterial blood pressure (manual syphygmomanometer) was checked every five minutes initially until all patients had achieved haemodynamic stability for at least 20 minutes.

A 7.5 F introducer (Edwards, Critical Care Division, Irvine, USA) was then placed in the right femoral vein under local anaesthesia (10 ml of 2% lignocaine). The hepatic venous pressure gradient (wedged hepatic venous pressure minus free hepatic venous pressure) was then estimated by inserting a Sidewinder II catheter (Cordis, USA) into the main hepatic vein under fluoroscopic screening. Inflating and releasing the balloon enabled repeated consistent measurements of wedge and free hepatic pressure respectively to be made. The Hewlett-Packard machine was zeroed for

Table 1 Baseline patient characteristics (Values mean (SEM))

Variable	Is-5-Mn, 10 mg (n=12)	Is-5-Mn, 40 mg (n=13)	p value
Mean age (y)	59.4 (3.3)	56.0 (2.3)	p>0.32 (NS)
Age range (y)	42-74	40-71	-
Sex (F/M)	8/4	3/10	-
Aetiology	10 ALC; 2 PBC	11 ALC; 1 PBC; 1 SBC	-
Child's-Pugh score	8.0 (0.6)	8.1 (0.6)	p>0.5 (NS)
Serum bilirubin (umol/l)	55.8 (16.0)	50.4 (12.9)	p>0.05 (NS)
Serum albumin (g/dl)	34.4 (1.7)	32.2 (1.7)	p=0.5 (NS)
PTR ratio	1.25 (0.08)	1.20 (0.05)	p=0.5 (NS)
Serum alanine aminotransferase (U/l)	36.2 (4.9)	32.6 (5.9)	p=0.5 (NS)
No with ascites	7/12	9/13	-

PTR=prothrombin ratio; ALC=alcoholic cirrhosis; PBC=primary biliary cirrhosis;
SBC=secondary biliary cirrhosis.

Table II Baseline haemodynamic characteristics. (Values, mean (SEM))

Variable	Is-5-Mn, 10 mg (n=12)	Is-5-Mn, 40 mg (n=13)	p value
Heart rate (bpm)	78.3 (3.5)	72.5 (3.0)	p>0.1 (NS)
Mean arterial blood pressure (mm Hg)	93.7 (4.0)	96.2 (2.2)	p>0.5 (NS)
Wedge hepatic venous pressure (mm Hg)	21.4 (2.0)	23.2 (1.6)*	p>0.317 (NS)
Free hepatic venous pressure (mm Hg)	5.5 (1.2)	7.1 (1.2)†	p>0.317 (NS)
Hepatic venous pressure gradient (mm Hg)	15.9 (1.8)	16.4 (0.7)*	p>0.5 (NS)
Azygos vein blood flow (ml/min)	378 (91)	511 (103)	p>0.317 (NS)
Liver blood flow (ml/min)	1227 (184)‡	1313 (159)§	p>0.5 (NS)

*n=12; †n=13; ‡n=5; §n=7.

pressure at the level of the right atrium at the start of each procedure.

Liver blood flow was measured by the constant infusion, indocyanine green dye method.¹⁸⁻¹⁹ The Sidewinder II catheter was then removed and an azygos catheter was introduced into the azygos vein under fluoroscopic screening. Estimation of the azygos blood flow was made by the thermodilution technique²⁰ using 5% dextrose at 50 ml/min as injectate. The position of the catheter was noted for the next occasion of its use in that patient to minimise the effect of change of catheter position on flow.

Either a 10 mg or a 40 mg tablet of Is-5-Mn was then administered orally. Heart rate, blood pressure, and azygos vein flow recordings were taken 30 minutes and 60 minutes after administration of the drug. Sixty minutes after administration of the tablet the azygos catheter was removed and the Sidewinder catheter was reinserted into the main right hepatic vein and a further estimation of wedge and free hepatic venous pressure and liver blood flow was made by the above methods.

CHRONIC ADMINISTRATION OF IS-5-MN

Each patient was given 100 tablets of their randomised dose of Is-5-Mn (Boehringer-Mannheim Ltd) and written instructions to take one tablet at 9 am and one at 5 pm each day – that is, allowing for a nitrate free interval. Each patient was asked to return the box of tablets at the end of the study (for a tablet count). The last morning dose (on the day of

the second invasive procedure) was omitted. The heart rate, blood pressure, hepatic venous pressure gradient, estimated liver blood flow, and azygos vein blood flow were measured again after 28 days of Is-5-Mn treatment both before and after rechallenge with their randomised nitrate dosage using the same methodology as above. Patients were asked to report headaches or any other side effect.

STATISTICAL ANALYSIS

Results are expressed as mean (SEM) haemodynamic values for each treatment group. Each mean variable at one time point was compared with the haemodynamic value at the time 0 (that is, baseline value) by a two tailed paired Student's *t* test.

Correlation of changes in each haemodynamic variable with serum albumin, bilirubin, and prothrombin ratio (PTR) was made using the Spearman rank correlation test on Systat Version 5.0 for Microsoft Windows.

Results

COMPLETION OF THE STUDY

Twelve patients were randomly allocated to receive 10 mg and 13 to receive 40 mg of Is-5-Mn. Four patients (two in each group) failed to attend for the second invasive procedure and in one patient the procedure was abandoned for technical reasons.

BASELINE HAEMODYNAMIC CHARACTERISTICS

No significant difference in baseline haemodynamic characteristics between the two treatment groups was seen (Table II).

HEART RATE RESPONSE TO IS-5-MN

No significant change in heart rate was seen acutely or after four weeks of 10 mg Is-5-Mn twice daily (Table III). In contrast, patients given 40 mg Is-5-Mn showed a significant rise in heart rate 30 and 60 minutes after drug administration which was still present after a month of therapy (Table III).

EFFECT OF IS-5-MN ON MEAN ARTERIAL BLOOD PRESSURE

Acute administration of either 10 mg or 40 mg Is-5-Mn produced a significant fall in blood pressure after 30 and 60 minutes (Table III). This fall in blood pressure was not sustained up to or after rechallenge with the nitrate at one month (Table III).

EFFECT OF IS-5-MN ON THE HEPATIC VENOUS PRESSURE GRADIENT

Wedge hepatic venous pressure fell significantly 60 minutes after 10 mg Is-5-Mn given initially and after one month of nitrate use (Fig 1). The pressure before challenge at one month, however, was not significantly different from the baseline (first study) value. A dose of

Table III Comparison of the effect of 10 mg and 40 mg Is-5-Mn on heart rate, mean arterial blood pressure (MABP), and azygos vein blood (AzygBF) flow in 25 patients with cirrhosis. (Values mean (SEM))

Variable	1st study t=0 min	1st study t=30 min	1st study t=60 min	After 1 month t=0 min	After 1 month t=30 min	After 1 month t=60 min
Dose	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg
Is-5-Mn/no	n=12	n=11	n=12	n=9	n=9	n=9
Heart rate (bpm)	78.3 (3.5)	79.0 (3.7)	80.5 (4.5)	75.8 (6.1)	77.3 (6.5)	77.4 (6.3)
MABP (mm Hg)	93.7 (4.0)	85.3* (4.5)	88.6* (4.5)	89.6 (4.8)	87.1 (4.4)	88.2 (5.2)
AzygBF (ml/min)	378.4 (91.2)	320.3* (91.2)	324.4 (71.9)	512.2 (130.0)	517.2 (152.7)	664.0 (142.4)
Dose	40 mg	40 mg	40 mg	40 mg	40 mg	40 mg
Is-5-Mn/no	n=13	n=13	n=13	n=11	n=11	n=11
Heart rate (bpm)	72.5 (3.0)	83.7* (3.6)	82.7* (3.6)	82.1* (4.6)	87.7* (5.0)	84.0* (5.7)
MABP (mm Hg)	96.2 (2.2)	87.8* (2.3)	89.2* (2.0)	94.4 (4.6)	89.6 (3.6)	90.3 (4.5)
AzygBF (ml/min)	511 (102.6)	448.5 (104.2)	445.3 (103.0)	384.7 (83.1)	391.0 (100.2)	491.8 (146.9)

*Denotes significant difference from 1st study t=0 (baseline value) at least at 95% level.

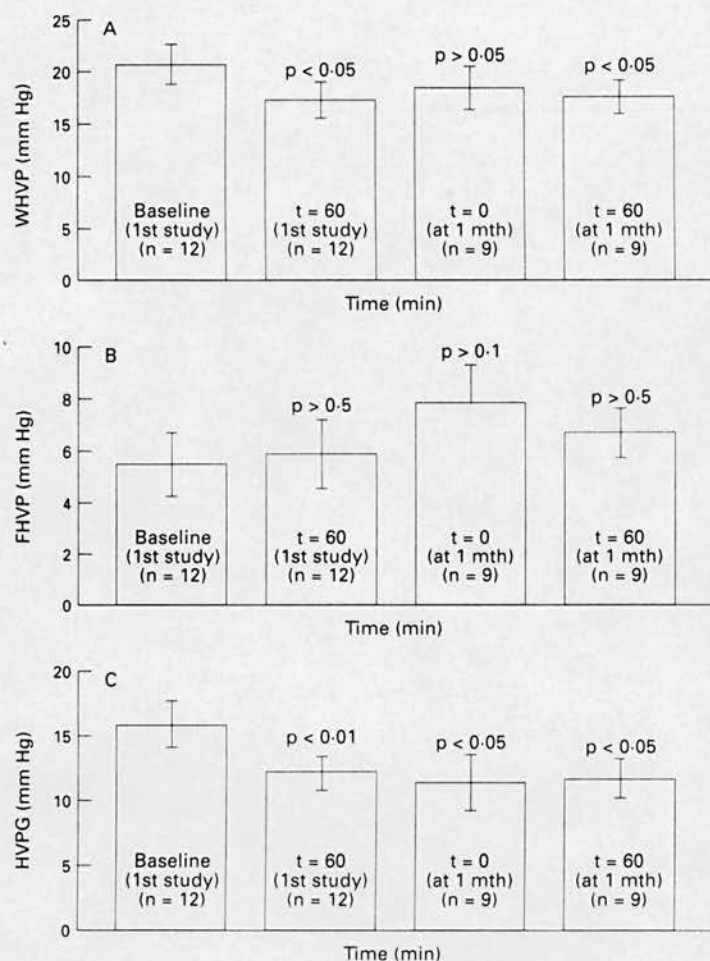


Figure 1: The acute and chronic effect of 10 mg isosorbide-5-mononitrate on mean wedged hepatic venous pressure (WHVP) (A), free hepatic venous pressure (FHVP) (B), and hepatic venous pressure gradient (HVPG) (C) in patients with cirrhosis. Mean values are given and error bars represent standard error of the mean and p values illustrate the result of paired t testing between values at the time points shown and the baseline (t=0) values.

10 mg Is-5-Mn had no significant effect on free hepatic venous pressure acutely or after chronic nitrate use (Fig 1). The hepatic venous pressure gradient showed sustained reduction after acute and chronic nitrate use (Fig 1).

The wedged hepatic venous pressure fell significantly acutely and after one month of 40 mg Is-5-Mn (Fig 2), an increasing effect being seen with more exposure to the nitrate. A dose of 40 mg Is-5-Mn had no statistically significant action on the mean free hepatic venous pressure (Fig 2). The hepatic venous pressure gradient showed sustained reduction after acute and chronic nitrate use (Fig 2), the greatest effect being seen on rechallenge with nitrate after a month of treatment.

EFFECT OF IS-5-MN ON AZYGOS VEIN BLOOD FLOW

A dose of 10 mg Is-5-Mn produced a significant reduction of azygos flow within 30 minutes of administration but thereafter no significant reduction was seen (Table III). Small reductions of azygos flow were seen acutely and chronically after 40 mg Is-5-Mn use but these failed to reach statistical significance (Table III). Most noticeable, however, was the variability of response of azygos blood flow to either dose of the nitrate as illustrated by the large standard error values. This can be

explained by division of all 25 patients (that is, both dosage groups) into arbitrary groups at baseline with low (less than 350 ml/min), moderate (350–550 ml/min), and high azygos flows (more than 550 ml/min). Although no statistical analysis between the groups would be valid, Figure 3 shows that those with low initial values increase in response to nitrate and those with high initial values reduce in response to nitrate. Those with intermediate values demonstrate little change in response to nitrate.

CORRELATION BETWEEN CHILD'S-PUGH VARIABLES AND RESPONSE TO IS-5-MN (SPEARMAN'S RANK CORRELATION TEST)

No significant correlation was found between the change in heart rate, mean arterial blood pressure, wedged hepatic venous pressure, free hepatic venous pressure, hepatic venous pressure gradient, and azygos blood flow with serum bilirubin, albumin, and prothrombin ratio in response to 10 mg Is-5-Mn at any time point in the first study or at one month.

Similarly, no correlations were found with the 40 mg group of Is-5-Mn except a reduction of free hepatic pressure (in the first hour, first study, $r = -0.72$) and reduction of azygos blood flow (in the first hour, second study, $r = 0.750$) were both significantly correlated ($p < 0.05$) with serum bilirubin. Also, the reduction in wedged hepatic venous pressure at the end of the second study hour (at one month) was correlated with serum albumin ($r = 0.65$). Azygos blood flow reduction from baseline to time zero on the first and second study were both correlated with serum albumin ($r = -0.633$ and -0.667 respectively). Only the reduction in free hepatic venous pressure after the first hour in the first study correlated with the prothrombin ratio ($r = 0.70$) in the 40 mg group.

EFFECT OF IS-5-MN ON ESTIMATED LIVER BLOOD FLOW (ICG METHOD)

Figure 4 shows that only 40 mg Is-5-Mn produces a significant fall in liver blood flow after one hour (which coincides with a drop in the mean arterial blood pressure). The liver blood flow did not show a significant reduction with chronic nitrate use.

SIDE EFFECTS

Three patients receiving 10 mg Is-5-Mn twice daily reported headaches during the first 24 hours of therapy. One of these patients described persistent headaches over the first two weeks and withdrew from the study.

Five patients reported headaches after starting 40 mg Is-5-Mn which stopped within 24 hours. One of these five patients experienced headaches for 10 days after beginning the tablets, lasting 10–20 minutes after each tablet, and withdrew from the study.

COMPLIANCE

Ten of the 10 mg group of patients (100%) of

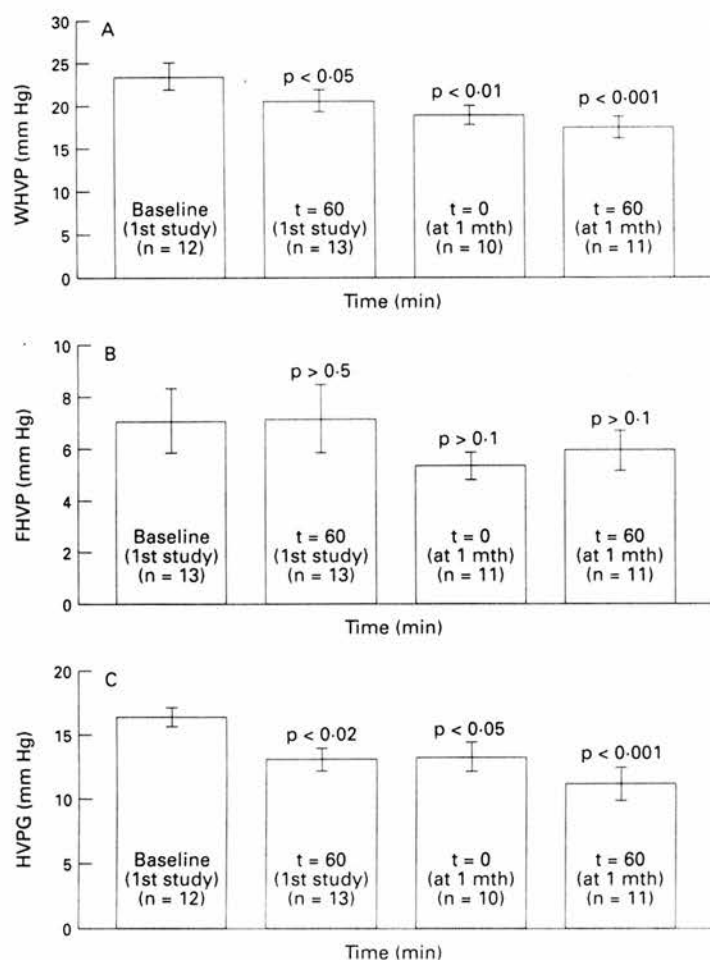


Figure 2: The acute and chronic effect of 40 mg isosorbide-5-mononitrate on mean wedged hepatic venous pressure (WHVP) (A), free hepatic venous pressure (FHVP) (B) and hepatic venous pressure gradient (HVPG) (C) in patients with cirrhosis. Mean values are illustrated, error bars represent standard error of the mean and p values illustrate the result of paired t testing between values at the time points shown and the baseline ($t=0$) values.

those attending the follow up haemodynamic study after one month) returned their unused tablets. Nine patients (90%) had returned the correct number of tablets and one patient had returned one tablet too many. All claimed to have taken their nitrate tablets diligently and had outpatient appointment attendance rates in excess of 85%.

Nine of the 40 mg group of patients (81.8% of those attending the follow up haemodynamic study after one month) returned their unused tablets. Eight patients (72.7%) had returned the correct number of tablets and the remaining patient had three tablets too many. Again all patients claimed to have taken their nitrate tablets and had outpatient appointment attendance rates in excess of 90%.

Discussion

Is-5-Mn is a powerful venous and mild arterial vasodilator.²¹ It has a prolonged half life (five hours),²² little first pass metabolism,²³ and dose linear kinetics²⁴ even in the presence of liver disease.^{25, 26}

NO EVIDENCE FOR DOSE-DEPENDENCY OF IS-5-MN IN REDUCTION OF HEPATIC VENOUS PRESSURE GRADIENT

Our study suggests that both 10 mg and 40 mg of Is-5-Mn greatly reduce the hepatic venous pressure gradient acutely and with chronic use. This is of clinical relevance given the increasing interest in the use of nitrates to prevent variceal bleeding in cirrhotic patients.

Only two previous groups have shown no effect of nitrates on hepatic venous pressure gradient.^{2, 14} All others showed a reduction in the hepatic venous pressure gradient which was accompanied by a fall in the cardiac index and mean arterial blood pressure (isosorbide dinitrate,¹⁻⁷ Is-5-Mn¹³⁻¹⁵). In our study there was no apparent advantage of one dose over the other in achieving portal pressure reduction.

HOW IS THE REDUCTION IN HEPATIC VENOUS PRESSURE GRADIENT ACHIEVED?

The reduction of hepatic venous pressure gradient in previous studies and ours (by either dose of Is-5-Mn) was achieved by a fall in the wedged hepatic venous pressure rather than a rise in the free hepatic venous pressure.

Reducing the wedged hepatic venous pressure may be more important than reducing the hepatic venous pressure gradient per se in protection against variceal bleeds.^{27, 28}

EVIDENCE FOR DIFFERENT DOSES WORKING BY DIFFERENT MECHANISMS

Many haemodynamic studies to date suggest that different doses of nitrate may act by different mechanisms^{15, 29} and this may well be the case in our study. Certainly, the 40 mg dose seems to induce a greater haemodynamic disturbance acutely – that is, increased heart rate, reduced mean arterial blood pressure, and liver blood flow – than the 10 mg dose. Only the increase in the heart rate effect was maintained after chronic use. Whether this means greater efficacy of the higher dose in prophylaxis of variceal bleeding remains to be evaluated by clinical studies.

HETEROGENEITY IN COLLATERAL VESSEL RESPONSIVENESS TO NITRATES

The efficacy of drugs in controlling an acute variceal bleed or preventing recurrent haemorrhage may be related to their ability to reduce blood flow through collateral vessels. Determination of the effects of vasoactive agents on both collateral blood flow and the hepatic venous pressure gradient is therefore important in evaluating their therapeutic potential.

Our results indicate that the effect of 10 mg or 40 mg Is-5-Mn on azygos blood flow was highly variable, including some individuals in whom flow increased suggesting vasodilatation of portasystemic collaterals. Those who had a low azygos flow increased their flow in response to nitrate and vice versa. Thus, the degree of pre-existing porta-systemic shunting seems important in the individuals' response to nitrate.

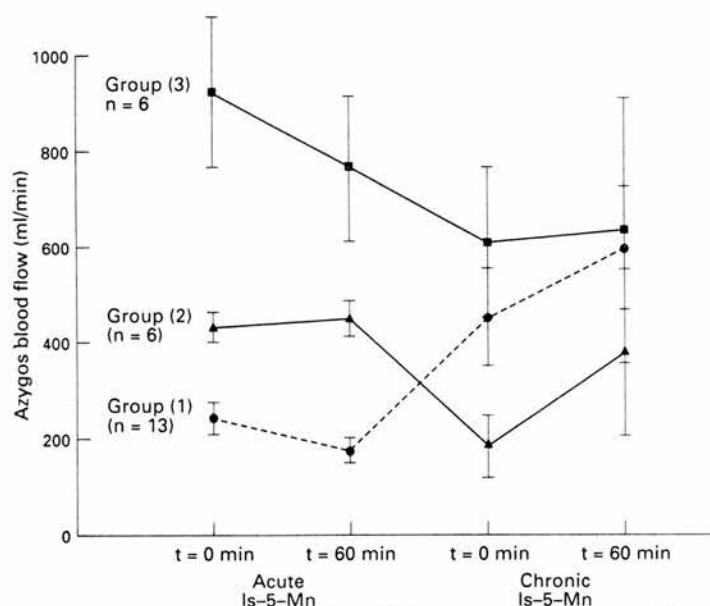


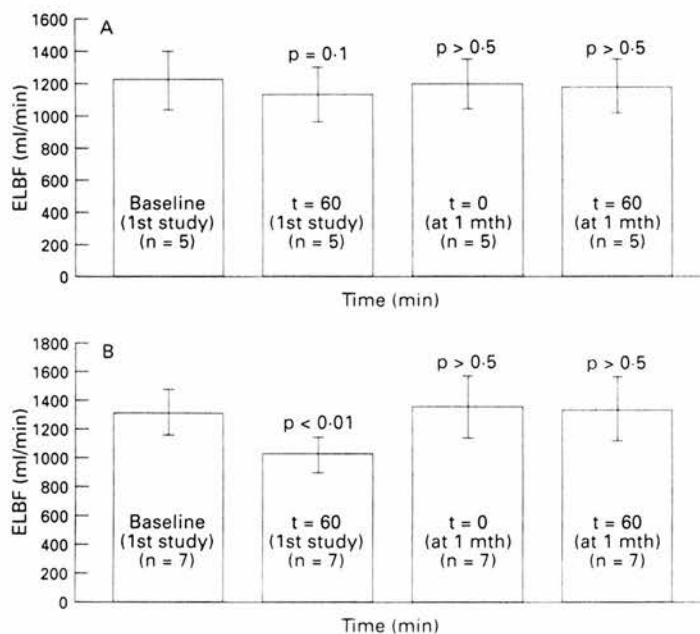
Figure 3: The acute and chronic effect of isosorbide-5-mononitrate administration (10 mg and 40 mg groups combined) on azygos blood flow in patients with cirrhosis. Patients are divided into 3 groups: (1) those with low initial azygos flows (<350 ml/min); (2) those with moderate azygos flows (350–550 ml/min); (3) those with high azygos flows (>550 ml/min). Mean azygos flows for each group are shown and the error bars represent standard error of the mean.

ROLE OF SEVERITY OF LIVER DISEASE IN PREDICTING AN INDIVIDUAL'S RESPONSE TO NITRATE

The severity of liver disease per se does not seem to be an important variable in defining the haemodynamic response to nitrates as so few haemodynamic changes correlated with serum bilirubin, albumin or prothrombin ratio (which are the best available, though imperfect, measures of severity). A few correlations tested positively but this would be expected by chance alone given the large number of correlations tested.

Other variables that have previously been suggested to be responsible for different

Figure 4: The acute and chronic effect of (A) 10 mg or (B) 40 mg isosorbide-5-mononitrate on liver blood flow (ELBF) estimated by the constant infusion method of indocyanine green. (Mean (SEM) values given.



response to nitrates between individuals may be the degree of autonomic impairment³⁰ or the baseline cardiac filling pressure.²⁹

TOLERANCE TO NITRATES

Tolerance is well recognised in patients with heart disease given nitrates for 18 hours or longer.^{31–32} Our study and others^{3–10–12–14–16} show a sustained fall in the hepatic venous pressure gradient after chronic nitrate administration, which suggests full tolerance is not of concern in patients with chronic liver disease. To postulate why this may be the case we need to first consider possible mechanisms of tolerance.

Multiple subcellular mechanisms of nitrate tolerance have been postulated, including conversion of the 'nitrate receptor' to the disulphide form with lower affinity for nitrate,³³ reduction of sulphhydryl groups necessary for the 'metabolic activation' of nitrates,³⁴ reduction of vascular production of nitric oxide from nitrate,³⁵ reduction in vascular metabolism to its dinitrate metabolites,³⁶ or molecular alteration of the cellular soluble guanylate cyclase.³⁷ These mechanisms explain in vitro phenomena, but are inadequate for the in vivo situation, for example nanomolar concentrations of nitrates cause tolerance in patients *versus* millimolar concentrations in vitro on blood vessels.³⁸

Recent evidence suggests, as expected, that both subcellular and physiological mechanisms are involved in nitrate tolerance in vivo.³⁹

Chronic nitrate administration is accompanied by a variety of compensatory physiological effects for example, raised plasma renin, catecholamines, body weight and sodium retention, and shifts in vascular volumes.^{40–42} These findings suggest that in vivo nitrate tolerance might be brought about by physiological compensatory mechanisms.

Patients with cirrhosis have profound physiological disturbance, however, including high plasma catecholamines, sodium retention, and shifts in vascular volumes,⁴³ and therefore may not be able to develop further compensatory mechanisms of tolerance.

EFFECT OF NITRATES ON LIVER BLOOD FLOW

Calculations of hepatic resistance from previous studies described above and our own study show that either acute or chronic administration of nitrates induce a significant reduction in resistance rather than a reduction in liver blood flow (which would be undesirable). This implies either that portal flow is not reduced or that a compensatory rise in hepatic arterial flow takes place, that is, reciprocity. The difficulty in distinguishing between these two inputs limits or ability to interpret these data further.

COMPLIANCE AND SIDE EFFECTS

Our data suggest that the patients were taking their nitrate tablets during this study; their motivation to attend outpatient clinics was high and the discrepancy in tablet counts minimal. Side effects were modest.

CONCLUDING REMARKS

An early clinical trial suggests a prophylactic benefit of nitrates for variceal bleeding,⁴⁴ although larger numbers of patients will be needed in future studies. Our work suggests that chronic administration of either 10 or 40 mg of Is-5-Mn, allowing for a nitrate free interval each day is effective in achieving hepatic venous pressure gradient reduction but there is no non-invasive way of predict collateral vessel response to nitrates.

Nitrates potentially have a number of different mechanisms of hepatic venous pressure reduction which may vary at different doses. Fortunately, by mechanisms which remain to be elucidated, patients with cirrhosis do not develop full tolerance to nitrates.

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